QUANTITATIVE GENETIC VARIATION INDUCED BY *P* TRANSPOSABLE ELEMENTS IN *Drosophila melanogaster*

By

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Declaration

I declare that this thesis is my own composition and that the research descibed herein has been done by me.

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CONTENTS

ABSTRACT

To determine the ability of the *P-M* hybrid dysgenesis system of *Drosophila melanogaster* to generate mutations affecting quantitative traits, *X* chromosome lines were constructed in which replicates of isogenic *M* and *P* strain *X* chromosomes were exposed to a dysgenic cross, a nondysgenic cross, or a control cross, and recovered in common autosomal backgrounds. Mutational heritabilities of abdominal and sternopleural bristle score were in general exceptionally high. *P* strain chromosomes were eight times more mutable than *M* strain chromosomes, and dysgenic crosses three times more effective than nondysgenic crosses in inducing polygenic variation. However, mutational heritabilities of the bristle traits were appreciable for *P* strain chromosomes passed through one nondysgenic cross, and for *M* strain chromosomes backcrossed for seven generations to inbred *P* strain females. The new variation resulting from one generation of mutagenesis was caused by a few lines with large effects on bristle score, and all mutations reduced bristle number. Among eight of these mutant lines and two additional mutant lines which occurred spontaneously during stock maintenance, the range of additive effects for both abdominal and sternopleural bristle scores are 2 to 9.5 bristles (1.2-5.5σ_p) in females, and 3.7 to 15.7 bristles (2.3-9.1σ_p) in males. The effects of nine mutant chromosomes (the exception is

line NDC(19)) on sternopleural bristle scores are similar and close to $1.3\sigma_p$, well within the wildtype range. The effects of mutants NDC(19) for both bristle traits, and lines DP(146) and DP(146)NAB for abdominal bristle scores, are extreme ($2.53\text{-}5.53\sigma_p$) compared with the wildtype. This result supports the hypothesis that the distribution of effects of mutant genes on quantitative characters is highly variable, and possibly leptokurtic. The overall degree of dominance of all mutant *X* chromosomes suggests they are partially recessive, but the effect of line NDC(19) on abdominal bristle score appears completely recessive.

Six of the mutant lines have similar low sternopleural bristle scores of about 15, are caused by allelic mutations with a map distance of approximately 24.7 cM, 4.7 cM from the *ct* locus. Deficiency mapping shows they are within chromosomal bands 8A4-8C6. Lines NDC(19) and DP(146) have extremely low abdominal and sternopleural bristle scores of 11.5 and 14.9, and of 10.3 and 12.5, respectively, and map closely linked to the *y* locus. The test of allelism and deficiency mapping indicate they are closely linked but at separate loci within chromosomal bands 1B2;1B4-6 and 1B4-6;1B10, respectively, with some epistatic effects. They are apparently single mutant alleles with pleiotropic effects on both bristle traits and viability. The mutation of line NDC(19) is probably at the *scute* locus. *In situ* hybridization analysis suggests these two mutants are associated with insertion of *P* elements within chromosomal region 1A-1B10, but the other six mutants probably resulted from excision of *P* elements at the site of 8C.

CHAPTER 1

INTRODUCTION

There is little need to elaborate the importance of quantitatively inherited characters in agricultural plants and animals since most economic traits belong to this category. The main efforts of modern plant and animal breeders are devoted to understanding the nature of the genetic variation underlying such characters. Most quantitative traits in most populations display heritable genetic variation. In theory quantitative genetic variation can be maintained by a balance of mutation and stabilizing selection, the level of which is determined by the mutation rate per locus, the number of loci contributing to the trait, the strength of stabilizing selection (Kimura, 1965; Latter, 1960, 1970; Lande, 1975; Fleming, 1979; Bulmer, 1972; Turelli, 1984; Keightley and Hill, 1988) and the phenotypic effect of mutation (Kimura, 1965; Lande, 1975; Fleming, 1979; Keightley and Hill, 1988). However, in domestic animals and cultivated plants, continual selection for "desirable characters" may limit the range of genotypes in the population, resulting in a continual elimination of genetic variability together with fixation of some relevant genes. In self-fertilizing crop plants particularly, the amount of genetic

variation present at a given time in a population and available to selection is very limited, which minimizes the effectiveness of artificial selection in these species.

Knowledge of the genetic nature of quantitative traits (i.e., the number of loci controlling a specified quantitative trait, the distribution of allelic effects, the pleiotropic and epistatic effects of/among loci) is still scanty. The answers to these questions are becoming increasingly important to breeders and biologists, who have been trying to increase efficiency of improvement of production traits of animals and plants and to fully comprehend the genetics of the evolutionary process.

The following is a summary of the current state of knowledge regarding these questions.

1.1 QUANTITATIVE MUTATIONAL VARIATION

1.1.1 Theory

How much quantitative variation can be maintained in natural and selected populations? Under the neutral theory, Clayton and Robertson (1955) first derived the equilibrium genetic variance for a quantitative trait within finite populations, in which the contribution from mutations is balanced by loss from genetic drift without selection. This theory was later extended by Lynch and Hill (1986) to include expected divergence between populations and dominance. The equilibrium level of total genetic variation is approximately $2NV_m$,

where N is the effective population size, and V_m is the new variation from mutation occurring each generation. The rate of increase of between-line variance per generation is ultimately $2V_m$ regardless of population size and the level of dominance of new mutations. However, the absolute amount of between-line variance at any time depends on the dominance properties of the new mutations. Fully dominant mutations contribute the greatest amount of variance, while recessive mutations contribute less variance than purely additive mutations. Similarly, during the approach to equilibrium the variance within populations is less for recessive and greater for dominant mutations, than for additive new mutants.

With regard to directional selection, Hill (1982a) established a model by which the magnitude of mutational variance arising from new mutations during selection can be estimated. The cumulated response (C_t) to t generations due to fixation of new additive mutations with a distribution of mutant effects symmetric about zero is a function of the selection intensity (i), population size (N), phenotypic standard deviation (σ_p) and the mutational variance (V_m), i.e., $C_t=2NiV_m/\sigma_p[t-2N(1-e^{-t/2N})]$. Then the estimate of mutational variance in generation t is $V_m=C_t\sigma_p/[(2Ni)(t-2N(1-e^{-t/2N}))]$. However, if new mutations affecting the selected quantitative trait have deleterious pleiotropic effects on fitness, the response to selection is less than expected from new neutral mutants, so V_m would be underestimated in this case. The variance maintained until fixation from mutants with deleterious fitness effects is less than for neutral mutations under directional selection.

The expected magnitude of genetic variance for a quantitative

trait maintained within a population when it reaches an equilibrium of mutation and stabilizing selection strongly depends on the model assumptions. The equilibrium genetic variance maintained in infinite and finite populations by the balance of mutation and stabilizing selection given different assumptions are summarized in Table 1.1. For an infinite population, based on Kimura's 'continuum of alleles' model, Lande (1975) assumed new mutations have effects that differ only slightly from those pre-existing, with an approximately normal distribution of allelic effects, and concluded that a high level of genetic variation can be maintained in the presence of strong stabilizing selection. However, under Turelli's model (1984), assuming that the effects of new mutations are likely to be larger than the existing range of variation at loci affecting the trait, with a non-normal distribution of allelic effects, the level of variance maintained is much less than predicted by the models of Kimura (1965) and Lande (1975). The variance maintained under Turelli's (1984) model depends only on the number of new mutants per generation, and is independent of the number and effects of alleles and the distribution of mutational effects. Under the models of Kimura (1965) and Lande (1975) the variance maintained depends on all of the above parameters.

The genetic variance in a finite population under the stabilizing selection model is usually less than that maintained in an infinite population since new mutations are subject to loss by drift as well as selection (Keightley and Hill, 1988). Unlike the infinite population case, the level of equilibrium genetic variance is influenced by both the scale and shape of the distribution of additive mutant effects. The mutants with a large effect which contribute most to mutational

variance do not contribute much to the equilibrium genetic variance, since selection against them is strong and they are quickly eliminated. Skewed distributions of mutant effects lead to lower levels of variance maintained in this model, because the population mean is displaced from the optimum and stabilizing selection is therefore stronger against most new mutants.

Maintenance of genetic variance in a population by a balance between mutation and stabilizing selection can also be affected by mutations with pleiotropic effects. For weak selection and small effects of uncorrelated mutations compared to segregating variance, significant genetic variance can be maintained if characters related by pleiotropy are jointly subject to stabilizing selection (Lande, 1980). Nevertheless, when selection is strong and the effects of new mutations are large compared to existing variation, pleiotropy between characters seriously reduces the equilibrium variance maintained for each trait (Turelli, 1985). Under pleiotropic models assuming that new mutations produce alleles which are only mildly deleterious, but have significant effects on the traits, genetic variation is likely maintained as the pleiotropic side effect of either deleterious mutation, or balancing selection (Barton, 1990). The strong stabilizing selection which is often observed is likely to be caused by correlations with a limited number of directly selected traits.

Although disparities are found among the quantitative predictions of the magnitude of genetic variance maintained for the different models, and they are sensitive to varying degrees to departure from model assumptions, all point to a potentially significant role for mutation in determining the amount of quantitative genetic variation in

TABLE 1.1 Predictions of Genetic Variance Maintained at Equilibrium Under Various Assumptions

Assumptions	Genetic variance maintained at equilibrium
Infinite population	
1. Kimura (1965) n mutationally equivalent loci small effects of new mutants weak stabilizing selection continuous-time	$V_g=\sqrt{(nV_m)}\sqrt{(2V_s)}$
2. Lande (1975) n mutationally equivalent loci small effects of new mutants weak stabilizing selection discrete-time	$V_g=\sqrt{(nV_m)}\sqrt{(2V_s+nV_m)}+nV_m$
3. Turelli (1984) n mutationally equivalent loci large effects of new mutants strong stabilizing selection	$V_g=4\gamma V_s$
Finite population	
4. Keightley & Hill (1988) n mutationally equivalent loci strong stabilizing selection	
a) sufficiently large effects of new mutants	$V_g=2NV_m/(1+NV_m/(2\gamma V_s))$
b) $\gamma\to\infty$ and infinitesimally small effects of mutants	$V_g=2NV_m$
c) increasing effects of drift ($Ns\to 0$)	$V_g=0$

$V_m = 2n\mu\sigma_a^2$, the rate of input of new genetic variance for n loci with mutation rate μ and the variance of mutational effects σ_a^2, where σ_a^2 is variance of mutant effect (a).

$V_s = w^2 + V_e$, where w is fitness of individual, and V_e is the environmental variance. V_s measures the intensity of selection on genotype and large V_s corresponds to weak selection.

γ: the number of new mutants affecting the trait per generation.

N: the effective population size.

populations. In addition, the neutral models (Lynch and Hill, 1986;Hill, 1982a,b) provide a theoretical basis from which to estimate quantitative mutational variation within and between lines and predict responses to selection from new mutations.

1.1.2 Observed Quantitative Mutational Variation

Drosophila melanogaster: Mather and Wigan (1942) initiated the first experiment demonstrating the accumulation of spontaneous mutations affecting bristle scores in *Drosophila melanogaster*. Divergent selection for sternopleural and abdominal chaeta number applied to a highly inbred population for 21 and 53 generations showed clear responses of 0.4 and 1.0 phenotypic standard deviation, respectively. By extending selection to include X-irradiated populations, Clayton and Robertson (1955) investigated the extent to which spontaneous and X-ray induced mutations cause genetic variance for bristle traits, and proposed the concept of mutational variance (V_m), the increment of genetic variance due to mutation in the population per generation, to quantify the rate of polygenic mutations. The general magnitude of mutational variance estimated from their experiment was roughly 10^{-3} times the environmental variance (V_e) for spontaneous mutations. This estimate is analogous to that (see Table 1.2) from selection for abdominal bristle scores by Mather and Wigan (1942) and from variance among second chromosomes extracted from an inbred strain by Durrant and Mather (1954). Subsequent estimates of spontaneous mutational variance from selection of inbred and plateaued selection lines by Clayton and Robertson (1964), and from an inbred base population by

Kitagawa (1967) and Hollingdale and Barker (1971) gave quantitatively similar results (see Table 1.2). However, the mutational variance estimated from the X-ray treated populations of Clayton and Robertson (1955, 1964), although slightly higher than the control (spontaneous) mutation rate, was small compared to the result of Scossiroli and Scossiroli (1959), who obtained large responses in sternopleural bristle scores by irradiating a nearly isogenic population of Drosophila. These large responses have not been repeated subsequently by other workers (Kitagawa, 1967; Hollingdale and Barker, 1971).

The theory of Clayton and Robertson (1955), Hill (1982a, 1982b) and Lynch and Hill (1986) has been used to calculate spontaneous and X-ray-induced mutational variance from response to selection for bristle scores of inbred lines. Spontaneous mutation rates for bristle traits average $10^{-3}V_e$ (Lande, 1975; Hill, 1982b). Mackay (1987) concluded that X-ray-induced mutations give on average $V_m=2.9\times10^{-3}$ V_e scaled to 1000r/generation, roughly three times higher than the spontaneous rate. Estimates of spontaneous polygenic mutation rate for a variety of species and characters have been summarized by Lynch (1988) (see Table 1.2). He showed that the mutation rate of quantitative traits (V_m/V_e) varies between populations, species, and characters with a range of approximately 10^{-4} V_e to 5×10^{-2} V_e. The mutation accumulation approach has also been extended to investigation of polygenes affecting fitness and viability of *Drosophila melanogaster*. Spontaneous lethal mutations on the second chromosome of Drosophila were observed to occur at a rate of 0.005-0.006 per chromosome per generation (Crow and Temin, 1964; Wallace, 1968; Mukai *et al*, 1972). However, quantitative mutational variance (V_m/V_e) for viability estimated in Drosophila (Mukai, 1964;

TABLE 1.2 Estimates of the Scaled Mutational Variance, V_m/V_e

Trait	Estimated V_m/V_e value					Reference
	Mean	Range	SD	n	M	
		Drosophila melanogaster traits				
Abdominal	0.0028	0.0006-0.0050	0.0022	3	S	Mather & Wigan 1942
bristles	0.0006	-	-	1	S	Clayton & Robertson 1955
	0.0003	0.0001-0.0009	0.0003	6	S	Clayton & Robertson 1964
	0.0034	0.0000-0.0102	0.0050	7	S	Clayton & Robertson 1964
	0.0006	-	-	1	S	Kitagawa 1967
	0.0007	-	-	1	S	Hollingdale & Baker 1971
	0.0016	0.0024-0.0041	0.0012	2	I	Durrant & Mather 1954
	0.0017	-	-	1	I	Paxman 1957
Sternopleural	0.0023	-	-	1	S	Mather & Wigan 1942
bristles	0.0034	0.0058-0.0077	0.0013	2	I	Durrant & Mather 1954
	0.0008	-	-	1	I	Paxman 1957
Viability	0.00004	-	-	1	I	Mukai 1964
	0.00003	0.00001-0.00006	0.00003	3	I	Mukai *et al.* 1972
	0.00001	-	-	1	I	Cardellino & Mukai 1975
	0.00002	-	-	1	I	Ohnishi 1977
ADH activity	0.0006	0.00059-0.00065	0.00004	2	I	Mukai *et al.* 1984
		Inbred lines of mice				
Skull traits						
metric	0.0046	0.0000-0.0118	0.0039	8	I	Bailey 1959
threshold	0.0057	0.0000-0.0426	0.0088	25	I	Carpenter *et al.* 1957,
					I	Deol *et al.* 1957,
					I	Yong 1972
Mandible measures	0.0231	0.00004-0.0716	0.0235	13	I	Festing 1973
Ilium length	0.0158	0.0084-0.0233	0.0105	2	I	Bailey 1959
Ulna length	0.0311	0.0175-0.0447	0.0192	2	I	Bailey 1959
		Vegetative and reproductive traits* in plants				
Barley	0.001	0.000-0.0004	0.0002	8	I	Cox *et al.* 1987
Corn	0.0078	0.031-0.0192	0.0054	5	I	Russell *et al.* 1963
	>0.0024	0.12-0.0034	0.0009	8	D	Sprague *et al.* 1960
Rice	0.0028	0.015-0.0038	0.0017	8	I	Oka *et al.* 1958
	0.0034	0.018-0.0056	0.0014	6	I	Sakai & Suzuki 1964

n: the number of replicates lines examined.
M: the breeding method used, S=response to selection from an inbred base population, I= divergence between lines from an inbred base population, and D= divergence between lines started from a double-monoploid base population.
*: see text for explanation.
After Lynch (1988)

Mukai *et al.*, 1972; Cardellino and Mukai, 1975; Ohnishi 1977a) is very low (see Table 1.2, re-estimated by Lynch, 1988). The low estimates area consequence of excluding mutations with large effects on viability. Clayton and Robertson (1964) found that 82% of third chromosome lines extracted from a Drosophila population that had received a cumulative dose of 270kr irradiation were homozygous lethal. One reason why irradiation has not been successful in generating significant genetic variation for quantitative traits is therefore the wide-scale genetic damage and induction of recessive lethals it causes. It takes a long time for individuals to recover physically from these treatments, and often those carrying mutations may be eliminated by stabilizing selection. So phenotypic selection applied to such a population is likely in the direction of unmutated genotype, rather than for new mutations. Simmons and Crow (1977) have reviewed the evidence for detrimental effects of X-irradiation and chemical mutagenesis on homozygous and heterozygous viability in Drosophila.

Plants and other animals: A long-term experiment of Russell *et al.* (1963) using several long-term inbred lines of corn provides data to estimate the spontaneous quantitative mutational variation for nine metric traits. Lynch (1988) estimated V_m/V_e for nine vegetative and reproductive characters which averaged 0.008 (Table 1.2). The estimate of V_m/V_e from other experiments (Sprague *et al.*, 1960) for the same traits is no less than 0.0024 (Lynch, 1988). Similar estimates were obtained for heading date and plant height of rice (Oka *et al.* 1958). However, the estimate from a recent experiment with three inbred lines of barley by Cox *et al.* (1987) is much lower, from 0.0000 to 0.0004.

Therefore, although the levels of mutational variability are of considerable importance in long-term experiments and evolutionary arguments, spontaneous mutation is not a significant factor in short-term selection.

Over the past forty years there have been a large number of experiments aiming at inducing beneficial mutational variation in agricultural plants by utilizing chemicals and irradiation (reviewed by Scossiroli, 1965; Gottschalk and Wolff, 1981). In peanuts, extensive work of Gregory (1955, 1956, 1956, 1957, 1961) showed that populations of normal seedlings from seeds treated with X-rays have greater variability for mean yield than untreated populations, and that phenotypic selection can accumulate positive mutations to create better strains. By using the same mutagens (Rawlings *et al.*, 1958) and neutrons (Humphrey, 1954) in soybean, the estimates of genetic variation for yield, plant height, maturity and seed size were increased on average to five times control level. In rice, after X-irradiation (6,000r and 12,000r) of seeds, Oka *et al.*, (1958) obtained a larger genetic component of phenotypic variation for heading date and plant height from treated seeds than the untreated seeds. However, the means of the two populations did not change. By employing various treatments of irradiation (thermal neutrons, X-rays and gamma rays) on different genotypes of *Triticum aestivum*, Borojevic and Borojevic (1968) created a substantial increase in the quantitative phenotypic variance of two traits, but the specific response to mutagenic treatment was not found in these traits in later generations. Similar results were reported by other workers using different crops (Ehrenberg *et al.*, 1965; Gaul, 1967; Scossiroli, 1964).

Attempts to induce mutations affecting quantitative traits in animals other than Drosophila have also been made. Roberts (1967) demonstrated a small increase in response for body weight after irradiation of a line of mice at a selection limit. Abplanalp *et al.* (1964) showed irradiated chicken sperm did not improve the response to selection for increased egg number.

Three main points are clear from these studies of effects of induced mutations on quantitative traits. 1) The estimates of phenotypic variation for different morphological or production traits are significantly increased in irradiated populations compared with non-irradiated populations. 2) The mean values for quantitative traits in irradiated populations are in most cases lower than in non-irradiated populations. 3) Subsequent selection on treated populations, particularly those which have been previously selected for a long time, in most instances showed little progress in improving the desired production traits. This has disappointed many workers who had expected mutagenesis to create the genetic variation of the traits and produce variants that could subsequently be selected in an improvement programme (Oka *et al.*, 1958; Yamagata, 1964; Matsuo and Onozawa, 1961; Sukai and Suzuki, 1964; Brock, 1967; Borojevic and Sesek, 1981).

1.2 UNDERSTANDING THE NATURE OF POLYGENES

Polygenes, or quantitative trait loci (QTLs), are defined as those loci at which segregation contributes to genetic variance associated with a continuous variable. Classical biometrical models of

additive, dominance and interaction components of variation of quantitative traits have provided plant and animal breeders with reasonably satisfactory methods of predicting response to artificial selection in the improvement of livestock and crops. However, our understanding of the nature and function of the individual genes conditioning the genetic component of variation in quantitative traits is still very poor.

The first question of interest is how many genes are involved in the control of most of the variance within a population or most of the difference between the means of two populations (Robertson, 1967). Mather and Jinks (1971) proposed that possibly hundreds of genes would be involved, while Thoday and Thompson (1976) argued that rather few loci were involved. The original method of determining the number of genes is the Castle-Wright index (Castle, 1922; Wright, 1952). This technique uses the means, μ_h and μ_l, and variances, σ_h^2 and σ_l^2, of two inbred strains with high and low values of the trait, and variances, σ_{F2}^2 and σ_{F1}^2 of their F_2 and F_1. The estimated number ($\tilde{n}$) of loci is given by

$$\tilde{n} = \frac{(\mu_h - \mu_l)^2}{8\ \sigma_s^2}$$

where $\sigma_s^2=\sigma_{F2}^2-\sigma_{F1}^2=\sigma_{F2}^2-(\sigma_h^2+\sigma_l^2)/2$. This method assumes equal gene effects, independent gene action, directional distribution of allelic effects, and unlinked loci. Wright (1959, 1968) suggested another index, $\tilde{n}=(\sigma_B^2-\sigma_h^2)/(\mu_B-\mu_h)$, for estimates of apparent gene number and for detecting a single major factor when a backcross generation is available. Lande (1981) stressed that Castle-Wright's method could also be used with

outbred populations and artificial selection lines (high and low) from a single base population.

A number of improvements of this method have been suggested by relaxing some of the assumptions (Falconer, 1989a; Mather and Jinks, 1982), or by estimating many more parameters (Lande, 1981; Cockerham, 1986). However, the effects of relaxing several assumptions simultaneously have not been investigated and the additional parameters are not usually available. When the assumptions are violated, the true number of loci will be substantially underestimated.

More reliable methods of estimating the number of genes influencing a quantitative trait involve mapping individual QTLs. The first attempt to map Drosophila bristle genes was made by Payne (1918), who found that a factor increasing scutellar bristle number of a selection line is situated on the X chromosome. Subsequently Karp (1936) succeeded in isolating chromosomal segments affecting abdominal bristle scores on the third chromosome by using a multiple recessive marker chromosome. He concluded there are at least six genes increasing or decreasing the bristle scores on the third chromosome.

A method of studying quantitative trait factors suggested by Sax (1923) is to track the factors by following their segregation with linked markers. Breese and Mather (1957) developed this method further by using a marker chromosome to divide up the third chromosomes of two selected lines (high and low) into six regions. Each of these six regions, consisting of known portions of two selected third chromosome, were tested for bristle effects. They showed at least six "factors" were involved in the third chromosome's contribution to the difference between the two selected lines.

By combining the method of Breese and Mather (1957) with that of Sax (1923), Thoday (1961) devised a more powerful technique. He and his colleagues first tested which chromosome contains polygenes affecting bristle traits of interest, and isolated these effective chromosomes. Then these chromosomes were tested against a marker chromosome with at least two recessive marker genes which are outside the polygene of interest and neutral with respect to their effect on the character. The recombinant progeny from the cross of a female heterozygous for tester and marker chromosomes to a homozygous marker male were scored and assigned by their phenotype into separate classes. The rest of the genome was assumed to be homozygous. In their series of studies, Thoday (1967, 1973) and Thoday and Thompson (1976) found that a total of nine major sternopleural bristle effects located on all three major chromosomes accounted for accelerated responses observed during selection.

A similar method was used by Davies (1971), who analyzed the lines of Davies and Workman (1971), and found a high sternopleural bristle third chromosome carrying at least eight increasing factors but no decreasing one. Combining Thoday's method with a more powerful progeny test, Shrimpton and Robertson (1988a,b) found a minimum of 17 "factors" responsible for an increase of 24 sternopleural bristles on a single third chromosome from a line of Drosophila selected for high scores of this trait. However, the practical use of this method depends on the availability of suitable markers, which should be neutral in effect on the character and close enough together so that the frequency of double crossovers between them is negligible. In addition, it requires careful progeny-testing to determine how many classes and

loci are involved; in general, the larger the sample sizes of the recombinant classes, the larger the number of loci that will be detected (MacMillan and Robertson, 1974). These requirements have restricted its application to *Drosophila melanogaster*.

An approach proposed by Wright (1952) involves backcrossing to a fixed genotype, and appropriate selection for segregation of dominant effects. This method was shown by Mostafa (1963) and Piper(1972) to be less powerful than that of Thoday (1961) because it isolates large chromosome sections rather than individual loci. Another method illustrated by Spickett *et al*. (1967) is to break down quantitative characters into their simpler discontinuous components, so the character becomes more easily handled and understood. This technique combined with that of Thoday (1961) and Shrimpton and Robertson (1988a,b) constitutes a powerful method for dissecting individual loci affecting a trait.

In plants, linkage analysis between Mendelian markers and quantitative traits started in the 1920's. Sax (1923) crossed two inbred bean lines (*Phaseolus vulgaris*), one having heavier pigmented seeds and the other being nonpigmented. He demonstrated that seed pigment is determined by a single locus with two alleles, P and p, linked to factors that act additively in increasing seed weight by 2.4 centigrams. Lindstrom (1924) discovered linkage between a locus for fruit colour and factors for fruit size in tomato. Similar approaches were reported in maize (Lindstrom, 1931), peas (Rasmusson, 1927), barley (Wexelesen 1933, 1934), and mice (Green 1931, 1933). However, this method is not generally practical because most phenotypic markers have a deleterious effect on the performed quantitative trait, and the

number of such markers is very limited in most species.

More recently, association with molecular markers such as isozymes or restriction fragment length polymorphisms (RFLPs) has been used to map QTLs in plants (Paterson *et al.*, 1988; Stuber, 1989). Given two inbred parents with alternative alleles at loci affecting the quantitative trait of interest, their progeny can be assayed for these alleles and genotyped individually with regard to the marker alleles. The different marker alleles represent specified chromosome regions which derive from one parent. In theory these chromosome regions can be isolated by genetic manipulation, and their effects on the expression of the quantitative trait assessed in a random or isogenic genetic background. This approach has been applied in corn (Frei *et al.*, 1986; Kahler, 1985; Edwards *et al.*, 1987; Stuber *et al.*, 1986, 1987) and tomato (Paterson *et al.*, 1988, Nienhaus *et al.*, 1987). Paterson *et al.* (1988) resolved quantitative traits into Mendelian factors by using a complete linkage map of RFLPs, and mapped six factors controlling fruit mass, four factors determining soluble-solids concentration and five factors conditioning pH located among nine chromosomes of tomato.

There are several limitations with this new method. First, not all quantitative traits will be amenable to dissection, because the method requires the two parents differ in phenotype by at least k environmental standard deviations, where k is the number of large effective factors conditioning the character. Second, this method is not able to distinguish between groups of tightly linked loci with small effects and a single locus with a large effect. Finally, the experiments are very expensive and time-consuming.

In addition to methods discussed above, other possible methods

for detecting major genes affecting quantitative traits have been proposed. These are well reviewed by Hill and Knott (1988).

The most direct method for mapping individual QTLs is by inducing mutations in them. However, attempts to induce mutations in quantitative traits by chemicals or X-rays have not been successful (see above section). Recently, transposable elements have been discovered to cause many spontaneous mutations in Drosophila, mice and maize (reviewed by Lambert *et al.*, 1988), and it has been suggested that insertional mutagenesis of transposable elements may be a novel method for both increasing genetic variance for quantitative traits and mapping QTLs (Mackay, 1984, 1985; Soller and Beckman, 1986). This method has the advantage that the QTL into which the transposable element has inserted can be cloned and analyzed at molecular level (Bingham *et al.*, 1981). The following sections review some general properties of transposable elements, the *Drosophila melanogaster P* element in particular, and initial results documenting generation of quantitative genetic variation by transposable element mutagenesis.

1.3 HYBRID DYSGENESIS AND *P* ELEMENT MUTAGENESIS IN DROSOPHILA

1.3.1 Transposable Elements and Hybrid Dysgenesis

Transposable elements (TEs) are a common component of the eukaryotic genome (reviewed by Finnegan, 1985). TEs are moderately repetitious sequences inserted at apparently random and dispersed

genomic locations, and have usually no obvious phenotypic effects.

A large number of transposable element families are known to exist in *Drosophila melanogaster*, and comprise about 10% of the whole genome (reviewed by Finnegan, 1985). Currently at least five families of TEs, including *P*, *I*, *F*, *copia*-like, and *foldback* factors, have been identified. Similar to transposable elements in other eukaryotic genomes, such as maize (Green, 1977), most TE families in Drosophila are chromosomally dispersed sequences (Young, 1979), and are usually embedded in non-repetitive DNA (Crain *et al.*, 1976). They can be mobilized in an active state. Observations of polymorphisms in the number and positions of several dispersed TE DNA sequences in Drosophila showed that the positions occupied by each TE family are highly variable between stocks and species (Ilyin *et al.*, 1978; Potter *et al.*, 1979). Recently, many spontaneous mutations in Drosophila, mice and maize (reviewed by Lambert *et al.*, 1988) have been shown to be caused by TEs. Also molecular studies (reviewed by Finnegan, 1985) have demonstrated that many Drosophila mutations are the results of DNA insertions, and there is a strong correlation between the genetic behaviour of an insertion mutation and the structure of the responsible transposable element. Some of these mutations are very unstable.

One fascinating phenomenon caused by TEs is hybrid dysgenesis - a syndrome of correlated genetic traits that occur among the progeny that result from crosses between certain Drosophila strains (Kidwell *et al.*, 1977). These traits can include point-mutations, chromosomal aberrations, temperature-sensitive sterility and male recombination. Three independently acting systems of hybrid dysgenesis in Drosophila have been described, the *P-M* (Kidwell, 1979), *I-R* (Breglino and

Kidwell, 1983), and the newly discovered *hobo* (Yannopoulos *et al.*, 1987; Blackman *et al.*, 1987) system. The *P*, *I* and *hobo* transposable element families are associated with the hybrid dysgenic traits.

In the *P-M* system, dysgenesis occurs when males of a paternally contributing (*P*) strain are mated with females of a maternally contributing (*M*) strain, but usually not from the reciprocal cross, nor from intra-*P* and intra-*M* strain crosses. Similarly, *I-R* dysgenesis is observed from a dysgenic cross of *I* strain males to *R* strain females, but not in the reciprocal nondysgenic interstrain cross, nor in intra-*I* and -*R* strain crosses (Kidwell, 1979). There are two main differences between these systems. First, the dysgenic traits are restricted to females in the *I-R* system but occur in both sexes in the *P-M* system. The second observable disparity between the two systems is the type of sterility induced. The type of female sterility in the *I-R* system is SF sterility (the failure of some eggs to complete embryonic development, Picard *et al.*, 1977) and it is highest at temperatures of 18-20°C. In the *P-M* system, the sterility is GD sterility (the failure of the gonads to develop in both male and female dysgenic flies) and it is highest at temperatures of 25-29°C.

The third hybrid dysgenesis system discovered recently in *Drosophila melanogaster* is associated with *hobo* elements, and results when males of an H strain, with *hobo* elements, are crossed to females of an E strain, lacking them (Blackman *et al.*, 1987). It causes temperature-independent GD sterility, but less is known of this system than for *P-M* and *I-R* dysgenesis.

1.3.2 *P* Element Mutagenesis

P-M hybrid dysgenesis is mediated by the *P* family of transposable elements, which are present usually in multiple copies in *P* strains, but are missing from *M* strains of *D. melanogaster* (Bingham *et al.*, 1982). There are two classes of *P* elements, nondefective or complete, and defective.

Complete *P* elements are 2907 bp in length and have one small and three large open reading frames (O'Hare and Rubin, 1983). These elements, sometimes referred to as *P* factors, seem to encode a transposase enzyme which is required to catalyze *P* element transposition (Bingham *et al.*, 1982), and can also be mobilized in response to the transposase produced by other intact *P* elements in the same genome.

Defective elements are abundant in most *P* strains. It appears that they are derived from the intact 2.9 kb elements by internal deletion of sequences (O'Hare and Rubin, 1983). These incomplete elements and those with frameshift mutations in any one of the three large open reading frames are incapable of encoding the postulated transpose enzyme. However, they may be mobilized by production of transposase from other intact *P* elements if they contain their 31bp inverted terminal repeats.

The first mutational trait analyzed was the GD sterility in the *P-M* system, which is expressed in the F_1 individual rather than in its

offspring. This trait is highly temperature-dependent. Dysgenic females are almost all sterile at 29°C (Kidwell and Novy, 1979), but are normally fertile at 24°C or below. The other important mutational trait is the hypermutable allele, weak singed (denoted sn^w), located in the *X* chromosome, which mutates at an extraordinarily high rate, sometimes exceeding 50% per generation in the dysgenic state (Engels, 1979). The property of sn^w mutability facilitates the test of *P-M* hybrid dysgenesis, because sn^w mutability is independent of temperature but is highly correlated with the frequency of temperature-dependent sterility (Kocur *et al.*, 1986). *P* element mutagenesis also causes chromosome breakage. Engels (1983) discovered the phenotype "heldup wing" (*hdp*) resulted from breakage of the *X* chromosome at position 17D on the salivary chromosome map, and that the sites of breakage are the locations of transposable elements.

What is the mechanism of *P* element mutagenesis? Many of the mutations arising from dysgenic crosses are unstable, suggesting that they are due to DNA insertions (Green, 1977; Golubovsky *et al.*, 1977; Engels, 1979; Simmons and Lim, 1980). Rubin (1982) proposed that *P* factors induce mutations by inserting into and disrupting genetic loci, or by imprecise excision causing deletion mutations or chromosome aberrations. This hypothesis has been repeatedly confirmed (e.g., Bingham *et al.*, 1982; O'Hare and Rubin, 1983), by demonstrating mutations induced by *P-M* dysgenic crosses result from insertions of *P* elements.

How frequent is *P* element transposition? In *M*♀♀ × *P*♂♂ dysgenic crosses, Bingham *et al.* (1982) estimated the frequency is 0.25 per element per generation. Similar estimates have been obtained

subsequently (Benz and Engels, 1984; Eggleston *et al.*, 1988). A much higher transposition rate of 1.82 per element per generation was observed when a transposase-producing *P* element called P[ry^+ Δ2-3] (99B) (see section 1.3.3) was utilized to mobilize defective *P* elements (Robertson *et al.*, 1988).

How does transposition of the *P* element occur? Hybrid dysgenesis arises only in the cross of males of the *P* strain to females of the *M* strain, so transposition of *P* elements must be at least partially associated with extrachromosomal factors. The condition in which *P* elements are mobilized is called *M* cytotype, while that repressing the activity of *P* elements is called *P* cytotype. In contrast to the comprehensive molecular understanding of the *P* element, cytotype is poorly understood. Engels (1984) provided evidence for the long-suspected notion that the cytotype determining factors is contributed by the *P* element itself. Engels and Preston (1984) demonstrated that an intact *P* element is sufficient to invoke the *P* cytotype. Very recently molecular genetic analysis (Dover *et al.*, 1987) has revealed a specific deletion-derivative (the *KP* element) which is able to repress *P* induced hybrid dysgenesis. The complete nature of the cytotype remains somewhat mysterious.

Relating transposition of *P* elements to cytotype, O'Hare and Rubin (1983) presented a repressor model that offers a way of understanding some of the preceding observations on the behaviour and inheritance of cytotype and activity of *P* factors. It is proposed that *P* factors encode at least two functions, a transposase and a regulator that can lead to the suppression of transposition. Only the intact *P* elements are capable of producing the *P* element transposase which is

required for transposition (Rio *et al.*, 1986). In the *P* cytotype, transposition is repressed by a high activity of regulator. In a dysgenic cross, *P* factor DNA is placed in the *M* cytotype, where there is neither transposase nor regulator, and expression of both functions of *P* factors results in transposition.

A contrasting titration model described by Simmons and Bucholz (1985) is based on the result of "transposase titration" experiments. This model proposes that the transposition frequency of a specific defective *P* element in the presence of an intact element could be reduced in proportion to the number of other defective *P* elements in the genome. Other mechanisms for the regulation of transposition by specific deletion derivatives (Voelker *et al.*, 1984; Daniels *et al.*, 1985; Sakoyama, 1985; Dover *et al.*, 1987) have been described.

1.3.3 The Application of *P* Element Mutagenesis

P element mutagenesis can be classified into two types, primary and secondary mutagenesis. Primary mutagenesis is usually caused by a *P* element insertion in a locus, which was previously free of *P* elements, or uninfluenced by other *P* element insertions. Secondary mutagenesis results from precise or imprecise excision of a *P* element which already resides in a locus or chromosomal region, or chromosomal rearrangements that have their breakpoints at, or approximately in, the end of existing *P* element insertions. As more and more evidence is being documented proving the practicability of *P* element mutagenesis, many advantages of *P* factors for genetic research have been confirmed.

Transposon tagging is a specialized application of primary

mutagenesis in which *P-M* hybrid dysgenesis is employed for induction of *P* element insertional mutations at a locus to be used as molecular markers for cloning. Bingham *et al.* (1981) proposed a novel method (called gene tagging) of studying any genetically defined locus at the molecular level, given a mutant of the gene of interest caused by insertion of a transposable element that itself has been cloned. The mutated gene flanking the inserted transposable element can be recovered from a DNA library by using the homology of the cloned transposable element with that inserted in the mutant. Element sequences can subsequently be removed from this clone by restriction endonuclease digestion, whereas the remainder can be used as a probe to hybridize with the unmutated normal gene, which then can be cloned and sequenced. This design has been applied successfully to the cloning and sequencing of the white locus of *D. melanogaster*, using a white mutant caused by insertion of the *copia* transposable element (Bingham *et al.*, 1981). However, there are multiple ways in which *P* elements can induce mutations, and the number of *P* elements in the genome of the progeny of hybrid dysgenic crosses is large, so it is tedious to deduce which insertion of the many present in the genome of individuals resulting from a dysgenic cross is responsible for the mutation of interest.

Recently Robertson *et al.*, (1988) have described the properties of a *P* element created by *in vitro* mutagenesis (Laski *et al.*, 1986). This element provides a stable genomic source of *P* element transposase. It carries a ry^+ marker, and has its 2-3 intron deleted. This element P($ry^+\Delta$2-3), has two useful properties: it is very active in making *P* transposase and so can catalyze nonautonomous *P* elements, but it makes no *P* repressor; and the element itself does excise at significant

frequencies when it is integrated at position 99B. Combining a stock containing P($ry^{+}\Delta 2\text{-}3$) with an "ammunition" stock containing abundant nonautonomous (defective) *P* elements can generate stable new insertions at high frequency (Robertson *et al*, 1988), and these mutations can be recovered efficiently and precisely. Many independent fly strains carrying single insertions at new locations have been generated recently using variations of this method (Cooley *et al.*, 1988; Bellen *et al.*, 1989). In this way gene tagging is more competent and specific.

The availability of cloned *P* factors enables DNA transformation using modified *P* elements in Drosophila (Spradling and Rubin, 1982; Rubin and Spradling, 1982). By utilizing sn^{w} to diagnose the presence of *P* factors, a cloned *P* factor was injected into germ cell region of *M* cytotype embryos. About half of the surviving adults expressed a singed mutation, indicating incorporation of *P* elements. It is also possible technically to combine a desired gene into a cloned *P* element and then inject the construct into *M* embryos. Three injected genes- *rosy* (Spradling and Rubin, 1983), *Dopa decarboxylase* (Scholnick *et al.*, 1983), and *Alcohol dehydrogenase* (Goldberg *et al.*, 1983) all function normally in spite of the location into which they are inserted in the genome. Meanwhile, use of Δ2-3 as the transposase source in transformation can increase the frequency of integration by eliminating the requirement for a helper element.

Overall, *P* element mutagenesis has broad usage in various aspects of genetic research. There are several advantages of *P* element mutagenesis compared with methods of irradiation and chemical mutagenesis: 1. High frequencies of primary mutations (insertion and

deletion) can be induced in many genomic regions. 2. Secondary mutations may occur at even higher frequencies than primary mutations and it offers an extremely efficient method of targeted mutagenesis when this type of mutation is appropriate. 3. The mutation rate is under genetic control and can be readily manipulated by making suitable crosses. 4. The use of P(*ry*$^{+}$ Δ2-3) and marked "ammunition" *P* elements provide an efficient and precise method for tagging and cloning a number of wild type genes. 5. In contrast to the use of irradiation and chemical mutagens, this method causes no direct physiological damage to the organism, is safe to human health, and no expensive specialized equipment such as X-ray machines or extraction hoods are required.

P element mutagenesis can be used to generate quantitative variation and identify QTLs. The evidence for this is described in the following section.

1.4 TRANSPOSABLE ELEMENT-INDUCED QUANTITATIVE VARIATION

Based on the nature of *P* element mutagenesis, Mackay (1984) inferred that mutations caused by *P* element transposition are likely to occur at loci controlling quantitative characters, and dysgenic (*M* ♀♀ × *P* ♂♂) and nondysgenic (*P* ♀♀ × *M* ♂♂) hybrids should differ genetically mainly due to *P* element activity in the former cross. If *P* element mutagenesis induces additive genetic variation for a quantitative character, artificial selection for that trait among

dysgenic hybrids would give a significant increased response compared with nondysgenic hybrids, and analysis of this response should lead to the estimation of the amount and nature of *P* element-induced quantitative variation.

In Mackay's first experiment (1984, 1985), both dysgenic and nondysgenic crosses were established to investigate the transposable element-induced response to artificial selection for abdominal bristle scores. Significant responses were obtained over ten generations of selection, and realized heritability, phenotypic variation, and additive genetic variation were all increased in dysgenic lines by factors of 2.4, 1.5, 2.5 and 3.9, respectively, relative to the nondysgenic control. She concluded that *P* element transposition in the dysgenic selection lines had induced additive mutational variation for abdominal bristle number which contributed to the increment of selection responses (Mackay, 1984, 1985). However, there are several limitations with this approach. First, variation arising from dysgenesis must be distinguished from variation segregating in the interstrain cross; second, it was discovered that transposition can occur in the nondysgenic cross, so the use of this cross as a control is imperfect; and finally, the two sets of hybrids from dysgenic and nondysgenic crosses are not exactly genetically identical, with respect to the derivation of *X* chromosomes.

A second experimental design was used to partially circumvent these problems (Mackay, 1986, 1987). Using appropriate chromosome markers and suitable crosses, *M* strain second chromosome lines contaminated with *P* elements were created. By taking a population of *M* strain second chromosome lines free of *P* elements as a control,

variance among contaminated second chromosomes was estimated to be increased relative to the control by a factor of 1.86 for fitness, 14.02 for viability, 2.43 for fertility, 1.37 for abdominal bristle number, 1.21 for sternopleural bristle number, and 1.63 for female productivity. The result also indicates insertion of *P* elements have an overall deleterious effect on homozygous fitness and its components. This is in agreement with the work of Yukuhiro *et al.* (1985), who showed a decrease in viability and accumulation of lethals on second chromosomes subjected to dysgenic crosses, and of Fitzpatrick and Sved (1986), who showed a decrease in fitness of chromosomes passed through a dysgenic cross.

Interestingly, a noticeable increment in variance of chromosomes subjected to the nondysgenic cross was observed in Mackay's (1986, 1987) second experiment. It may suggest that transposition also occurred with appreciable frequency in the progeny of the nondysgenic cross. By extending Mackay's experimental design (1985) to include the independent *I-R* system of hybrid dysgenesis, Pignatelli and Mackay (1989) showed that *P-M* and *I-R* dysgenesis appear to be equally effective in creating mutational variance for two quantitative traits. However, although some selection lines gave large responses suggestive of new mutations, these did not occur in all lines, and lines with accelerated responses were descended from both dysgenic and nondysgenic crosses. Similar results were observed by Torkamanzehi *et al.* (1988), who repeated Mackay's (1985) experimental design. The hypothesis that transposition of *P* elements ultimately occurs in the populations found from nondysgenic crosses may be caused by segregation of *P* elements in the F_2 and subsequent generations, leading to a switch from initial *P*

cytotype of nondysgenic individuals to *M* cytotype in later generations (Mackay, 1987). This hypothesis is supported by the molecular analysis of her selected lines, in which similar number of "new" insertion sites of *P* elements were detected in both dysgenic and nondysgenic selection lines (Shrimpton *et al.*, 1990). The differences among experimental results also may be due to the diversity of *P* strains used, in terms of the positions and numbers of *P* elements residing near the loci affecting bristle traits.

In conclusion, there is experimental evidence that a significant amount of new variation affecting quantitative traits can be generated by *P* element mutagenesis, and the effect of *P* element mutagenesis in producing polygenic variation is very much greater than that of X-irradiation and chemical treatments. Mackay (1985) calculated the new variation for abdominal bristle score arising from dysgenesis is equivalent to that generated by irradiation at a cumulated dose of 750,000r. However, the results of other workers are less clear-cut. There is a need to improve the procedure for generating *P*-induced polygenic mutations by limiting transposition to a single generation, and using a highly inbred chromosome on which to accumulate mutations, both to estimate more precisely the *P*-induced polygenic mutation rate and to create material suitable for detailed genetic and molecular analysis. The results of such an experiment are presented in the following chapters.

CHAPTER 2

HYBRID DYSGENESIS-INDUCED QUANTITATIVE VARIATION ON THE *X* CHROMOSOME OF *Drosophila melanogaster*

2.1 INTRODUCTION

To determine the extent to which *P* element-induced mutations can cause variation for quantitative traits, it is necessary to compare the amount of genetic variation for the traits of interest in strains in which *P* elements have and have not moved, but which otherwise have a common genetic background. The difference in amount of genetic variance between these strains estimates the *P* element-induced mutational variance (V_m), from which the mutational heritability ($\hat{h}^2_m = V_m/(V_m + V_e)$, where V_e is the environmental variance) can be derived. Hybrid dysgenesis-induced *P* element mutagenesis has been used successfully to mutate and subsequently clone many major genes (reviewed by Kidwell, 1986). However, application of *P-M* hybrid dysgenesis to the generation of polygenic mutations has proven rather less straightforward than generating mutations of large qualitative effect (see Chapter 1). *P*-induced mutants of large effect are readily observed in the F_2 progeny of dysgenic hybrids, but polygenic mutations of smaller effect are likely to be swamped by the variance for the trait segregating in the F_2 as a result of the interstrain cross. *P* strain chromosomes may be more susceptible to *P*-induced mutations than

M strain chromosomes because excisions and rearrangements as well as transpositions can occur on the former, but only transposition will cause mutations in the latter. The process of placing the mutagenized chromosomes in a common background by backcrossing to a *P* or *M* strain has the advantage of allowing the detection of mutations of smaller effect than can be detected if the background remains segregating, but further mutations may occur on the target chromosomes during backcrossing.

To determine the effects of different procedures for generating chromosome lines contaminated with *P* elements on hybrid dysgenesis-induced polygenic mutation rates, I have constructed *X* chromosome lines from crosses of inbred *P* and *M* strains that allow me to compare mutational variance of two bristle traits arising in one generation on *M* strain and *P* strain *X* chromosomes after a dysgenic or a nondysgenic cross. In addition, the effect on mutational variance of homogenizing the genetic background by seven generations of backcrossing to either inbred *P* or *M* strains was studied for *M* strain *X* chromosomes that were passed through a single dysgenic or a single nondysgenic cross.

2.2 MATERIALS AND METHODS

2.2.1 Drosophila Stocks

The gene markers and chromosomes used are described in Lindsley and Grell (1968). All flies were reared on 10 ml agar-yeast-glucose

medium in shell vials.

1. Inbred Samarkand (Sam). This is a long-established laboratory strain, originally obtained from R. Middleton. It is classified as pure *M* in the *P-M* system of hybrid dysgenesis, as it contains neither complete nor defective *P* elements, and *I* in the *I-R* system of hybrid dysgenesis. A single subline of this strain was maintained for 75 generations by continuous full-sib inbreeding, followed by several generations of random mating in small mass cultures. It was further inbred by 19 generations of full-sib mating prior to the commencement of the experiments described below, then inbreeding was again relaxed for several generations to amplify the stock.

2. *C(1)DX y w f/*$_{Y}$; Sam (Sam attached-*X*). An attached-*X* stock in which the free *X* and *Y* chromosomes and the autosomes were derived from the inbred Samarkand strain by 10 generations of backcrossing attached-*X* females to Sam males. Male progeny of attached-*X* females receive their *X* chromosome from their father and their *Y* chromosome from their mother.

3. Inbred Harwich. A sample of the Harwich strain used was obtained originally from M. Kidwell. It is a strong *P* strain in the *P-M* system of hybrid dysgenesis, with an average copy number of approximately 40 *P* elements per individual (Shrimpton *et al.*, 1990), and is classified as *I* in the *I-R* system of hybrid dysgenesis (Kidwell, 1979). A single subline of this strain was maintained by 41 generations of continuous full-sib inbreeding, which was relaxed for several generations to build up numbers before it was used in the experimental crosses.

4. *C(1)DX y w f/*$_{Y}$; Harwich (Harwich attached-*X*). An attached-*X*

stock in which the free *X* and *Y* chromosomes and the autosomes were derived from the inbred Harwich population by 10 generations of backcrossing attached-*X* females to Harwich males. (As these were initially dysgenic crosses, the first five backcross generations were reared at 20°C to avoid sterility.)

All strains described above were kindly provided by Dr. Trudy F.C Mackay.

2.2.2 Derivation of *X* Chromosome Lines

The following crosses were designed to estimate the mutational variance of quantitative traits induced on inbred *P* strain and *M* strain *X* chromosomes passed through single dysgenic (*M* ♀♀ x *P* ♂♂) or nondysgenic (*P* ♀♀ x *M* ♂♂) crosses, while maintaining a common autosomal background. All crosses were made at 20°C. These crosses are depicted diagrammatically in Figure 2.1.

(1) Dysgenic cross, *M* strain *X* chromosome (N=100 lines): Inbred Sam (*M*) females were crossed to inbred Harwich (*P*) males in small mass matings of eight males and eight females. Each chromosome line was founded by a single dysgenic F_1 male, which was crossed to five Sam attached-*X* females. F_2 males inherit copies of their father's *M* strain-derived *X* chromosome which was potentially contaminated with *P* elements from the dysgenic cross. The autosomal background of the F_2 males segregates for Sam (75%) and Harwich (25%) alleles and may also be heterozygous for autosomal mutations induced by the dysgenic cross. A second replicate of N=148 lines was made of this cross (see below).

(2) Nondysgenic cross, *M* strain *X* chromosome (N=94 lines): Attached-*X* Harwich (*P*) females were crossed to inbred Sam (*M*) males in small mass matings of eight males and eight females. Each chromosome line was founded by a single F_1 nondysgenic male, which was crossed to five Sam attached-*X* females. F_2 males inherit copies of their father's *M* strain-derived *X* chromosome that passed through a nondysgenic cross, in an autosomal background that segregates for Sam (75%) and Harwich (25%) alleles.

(3) Dysgenic cross, *P* strain *X* chromosome (N=97 lines): Attached-*X* Sam (*M*) females were crossed to inbred Harwich (*P*) males in small mass matings of eight males and eight females. Each chromosome line was founded by a single dysgenic F_1 male crossed to five Sam attached-*X* females. F_2 males inherit copies of their father's *P* strain-derived *X* chromosome which was potentially contaminated with *P* elements from the dysgenic cross, in an autosomal background identical to that of cross (1) above.

(4) Nondysgenic cross, *P* strain *X* chromosome (N=96 lines): Inbred Harwich (*P*) females were crossed to inbred Sam (*M*) males in small mass matings of eight males and eight females. Each chromosome line was founded by a single nondysgenic F_1 male crossed to five Sam attached-*X* females. F_2 males inherit copies of their father's *P* strain-derived *X* chromosome that passed through a nondysgenic cross, in an autosomal background identical to that of cross (2) above.

(5) Dysgenic control (N=126 lines): Inbred Sam (*M*) females were crossed to inbred Harwich (*P*) males in small mass matings of eight males and eight females, and single dysgenic F_1 males were crossed to five inbred Sam females to found each chromosome line. Germ-line *P*

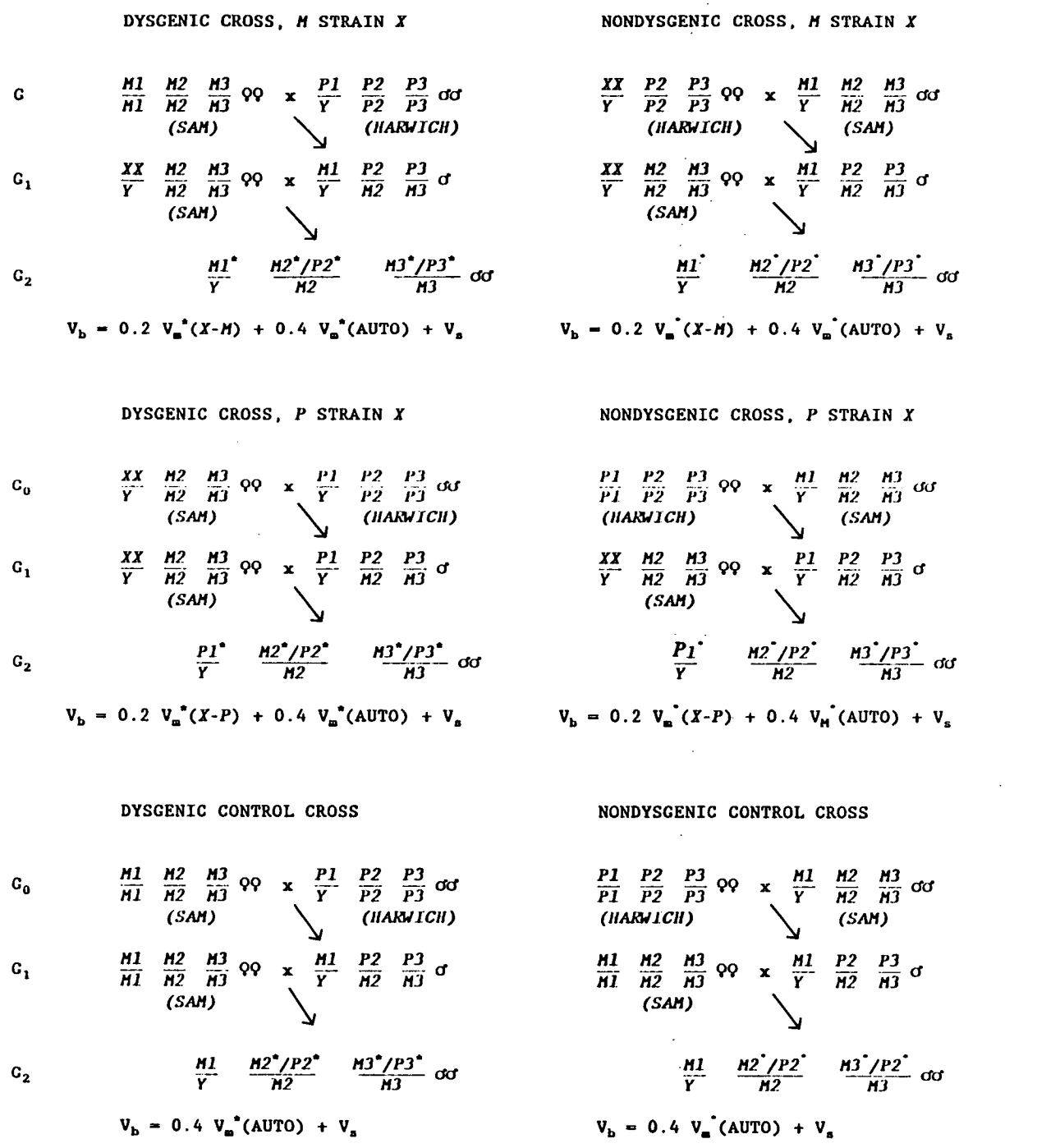

Figure 2.1 Crossing schemes to generate *P* and *M* strain *X* chromosome lines from dysgenic and nondysgenic crosses, and control *M* strain *X* chromosome lines, in a common autosomal background, and the expected composition of variance components between these lines (see text for explanation).

element transposition may occur on the *M* strain-derived *X* chromosomes of the F_2 males, but these mutations will not be expressed somatically. The autosomal background is identical to that of crosses (1) and (3) above.

(6) Nondysgenic control (N=121 lines): Inbred Harwich (*P*) females were crossed to inbred Sam (*M*) males in small mass matings of eight males and eight females, and single nondysgenic F_1 males were crossed to five inbred Sam females to found each chromosome line. Mutations caused by germ-line *P* element transposition on the *M* strain-derived *X* chromosomes of the F_2 males are not expressed somatically, and the autosomal background is identical to that of crosses (2) and (4) above.

Quantitative characters: Two quantitative traits, abdominal and sternopleural chaeta score, were measured on 20 F_2 males from each of the *X* chromosome lines (634 chromosome lines in total). Abdominal chaeta score was measured as the number of bristles on the posterior abdominal sternite, and sternopleural bristle score was the sum of the number of sternopleural bristles on the right and left sternopleural plates. All progenies scored for each line came from a single vial.

2.2.3 Expected Composition of Between-line Variance Components

The source of *X* chromosomes in each of the sets of chromosome lines described above is from a highly inbred strain, therefore the expected component of variance of bristle scores between lines contributed by the *X* chromosome is zero in the absence of mutation. However, the autosomes in each case segregate for alleles of the Sam strain and the Harwich strain, and this segregational variance (V_s) will

cause divergence among line means. Additional variance between the lines may arise from new polygenic mutations affecting bristle score on the *X* chromosomes and the autosomes as the result of either dysgenic or nondysgenic crosses. Let V_m be the mutational variance per generation accruing from a dysgenic (superscript *) or nondysgenic (superscript ^{0}) cross. (Although two generations are required to synthesize each line, only *P* element-induced mutations occurring in the germline of F_1 hybrids are expressed somatically in F_2 individuals, so mutational variation produced in one generation is measured). Let V_m(auto) refer to autosomal mutational variation and $V_m(X\text{-}M)$, $V_m(X\text{-}P)$ refer to mutational variance produced on *X* chromosomes of *M* and *P* strain origin, respectively. The expected composition of the between -line variance component (V_b) for each set of chromosome lines is then as follows.

(1) Dysgenic cross, *M* strain *X* chromosome: The variation between lines is caused by segregational variance, V_s, and mutational variance on the *X* chromosome and autosomes as a result of the dysgenic cross ($V_m^*(X\text{-}M)$ and V_m^*(auto)). The *X* chromosome is approximately 20% of the genome, so the proportion of variance contributed by new variation on the *X* is 0.2 $V_m^*(X\text{-}M)$. The autosomes constitute the remaining 80% of the genome, but mutations on the autosomes are heterozygous in F_2 males, so additive mutational variation contributed by the autosomes is 0.8 V_m^*(auto)/2. Thus $V_b = 0.2\ V_m^*(X\text{-}M) + 0.4\ V_m^*(\text{auto}) + V_s$.

(2) Nondysgenic cross, *M* strain *X* chromosome: These lines differ from (1) only in the direction of the original interstrain cross. Therefore $V_b = 0.2V_m^\circ(X\text{-}M) + 0.4\ V_m^\circ(\text{auto}) + V_s$.

(3) Dysgenic cross, *P* strain *X* chromosome: These lines differ from (1) only in the source of *X* chromosome. Therefore $V_b = 0.2\ V_m^*\ (X\text{-}P) + 0.4$

V^* (auto) + V_s.

(4) Nondysgenic cross, *P* strain *X* chromosome: These lines differ from (2) only in the source of *X* chromosome. Therefore $V_b = 0.2\ V_m^\circ(X\text{-}P) + 0.4\ V^\circ(\text{auto}) + V_s$.

(5) Dysgenic control: The *X* chromosomes of the F_2 males of this cross come directly from the inbred Sam (*M*) strain, and therefore contribute no variation between lines. However, the autosomes are heterozygous for any mutations occurring as a result of the dysgenic cross. Therefore $V_b = 0.4\ V_m^*(\text{auto}) + V_s$.

(6) Nondysgenic control: These lines differ from (5) in the direction of the initial cross. Therefore $V_b = 0.4\ V_m^\circ(\text{auto}) + V_s$.

2.2.4 Estimation of Mutational Variance

Components of variance between lines for the two quantitative traits ($\hat{V}_b$) were estimated by the least squares and maximum likelihood analysis of variance programme of W.R. Harvey. Estimates of mutational variance ($\hat{V}_m^*(X\text{-}M)$, $\hat{V}_m^*(X\text{-}P)$, $\hat{V}_m^\circ(X\text{-}M)$, $\hat{V}_m^\circ(X\text{-}P)$) were obtained by equating observed and expected between-line variance components, and solving the simultaneous equations for crosses (1)-(4) with the average between-line variance of the control crosses.

2.2.5 Evolution of Mutational Variance

At generation F_3, dysgenic, *M* strain *X* chromosome lines and

nondysgenic, *M* strain *X* chromosome lines were chosen at random to be backcrossed to either inbred Sam (*M*) or Harwich (*P*) strains. For seven generations, five males from each line were crossed to five virgin females from either the Sam attached-*X* or Harwich attached-*X* strain. At the end of the backcrossing period the autosomes are expected to be nearly isogenic (greater than 99% identity (Falconer, 1989a)) with the inbred backcross parent strain, although there may be residual heterozygosity remaining from autosomal mutations occurring during backcrossing. The variance between these lines for the bristle traits therefore is due mainly to accumulated mutational variation on the initially isogenic *X* chromosomes. Comparison of the variance components between lines for the two traits from the backcross lines to the between-line variance components after a single dysgenic or nondysgenic cross will indicate to what extent the mutational variance has altered over time, and will provide data on mutational heritability comparable to that of Mackay (1987). A total of 28 nondysgenic, *M* strain *X* chromosomes were backcrossed to Sam attached-*X* females, and 30 to Harwich attached-*X* females; 46 dysgenic *M* strain *X* chromosomes were backcrossed to Sam attached-*X* females and 39 to Harwich attached-*X* females.

2.3 RESULTS

The distributions of sternopleural and abdominal bristle score for chromosome lines derived from inbred *M* and *P* strain *X* chromosomes passed through a single dysgenic cross, a single nondysgenic cross, or a control cross, and recovered in a common autosomal background, are

shown in Figures 2.2 and 2.3. The statistical analyses of these distributions are given in Table 2.1.

2.3.1 Population Means

The overall means of the dysgenic, nondysgenic, and control populations of *M* strain *X* chromosomes differ little for either trait. However, average bristle scores of the *P* strain *X* chromosomes are lower for dysgenic than nondysgenic populations, and also lower than mean bristle scores of *M* strain *X* chromosome populations. The latter difference is because inbred Harwich (*P*) has lower average bristle scores for both traits than inbred Sam (*M*), and alleles contributing to the difference are present on the *X* chromosome. The magnitude of the effect contributed by *M*-derived *versus* *P*-derived *X* chromosomes can be calculated by comparing mean bristle scores of F_1 males from dysgenic and nondysgenic crosses of inbred Harwich and Sam with *M* strain *X* chromosomes (mean abdominal bristle score, 20.0; mean sternopleural bristle score, 19.0) to average bristle scores of F_1 males from dysgenic and nondysgenic crosses of these strains with *P* strain *X* chromosomes (mean abdominal bristle score, 18.1; mean sternopleural bristle score, 17.6). This difference of 1.9 abdominal and 1.4 sternopleural bristles contributed by *M* and *P* strain *X* chromosomes is consistent with that observed between the populations of F_2 males with *P* *X* chromosomes and those with *M* *X* chromosomes. The significant ($p<0.02$) difference of 0.6 abdominal and 0.7 sternopleural bristles between *P* strain *X* chromosomes derived from nondysgenic compared to dysgenic crosses appears to be caused by several lines with extremely low bristle scores in the *P* *X*

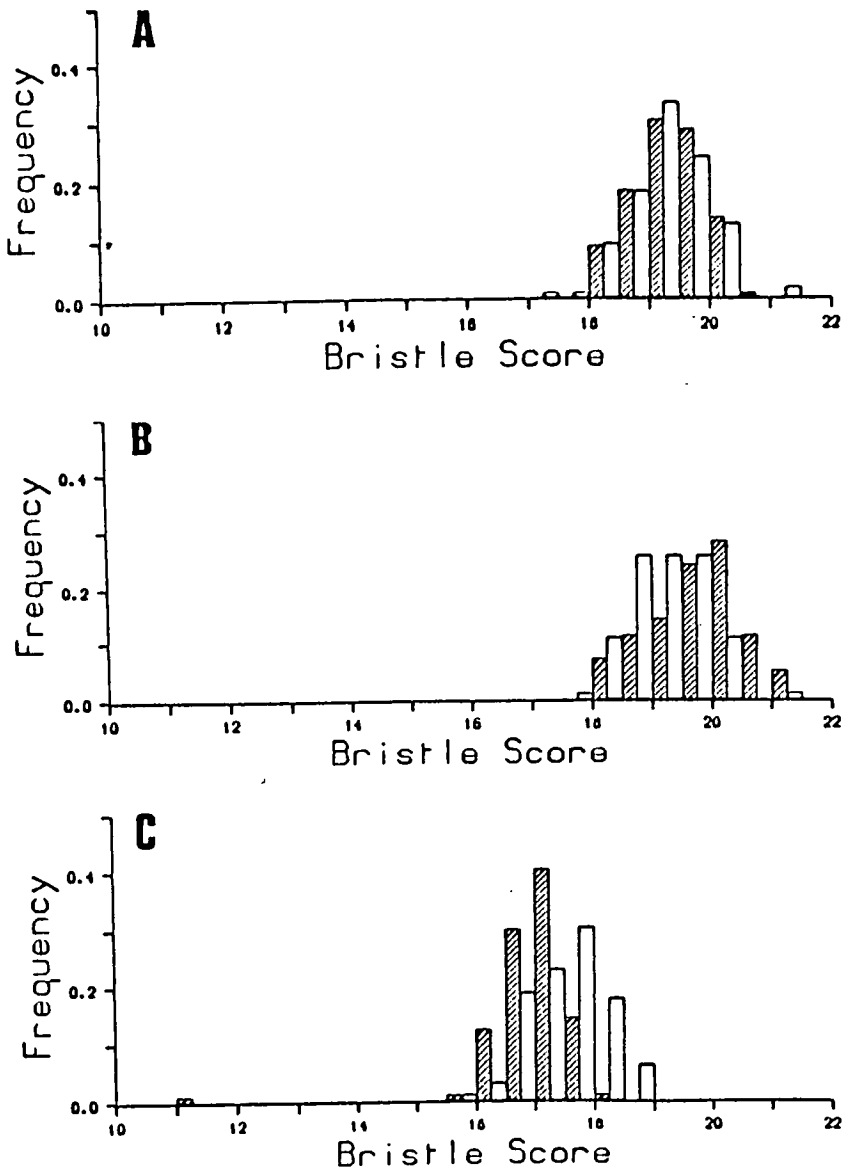

Figure 2.2 Distributions of *X* chromosome line means for abdominal bristle score. Dysgenic (cross-hatched bars) and nondysgenic (open bars) control *M* strain *X* chromosomes are shown in panel A, dysgenic and nondysgenic *M* strain *X* chromosomes in panel B, and dysgenic and nondysgenic *P* strain *X* chromosomes in panel C.

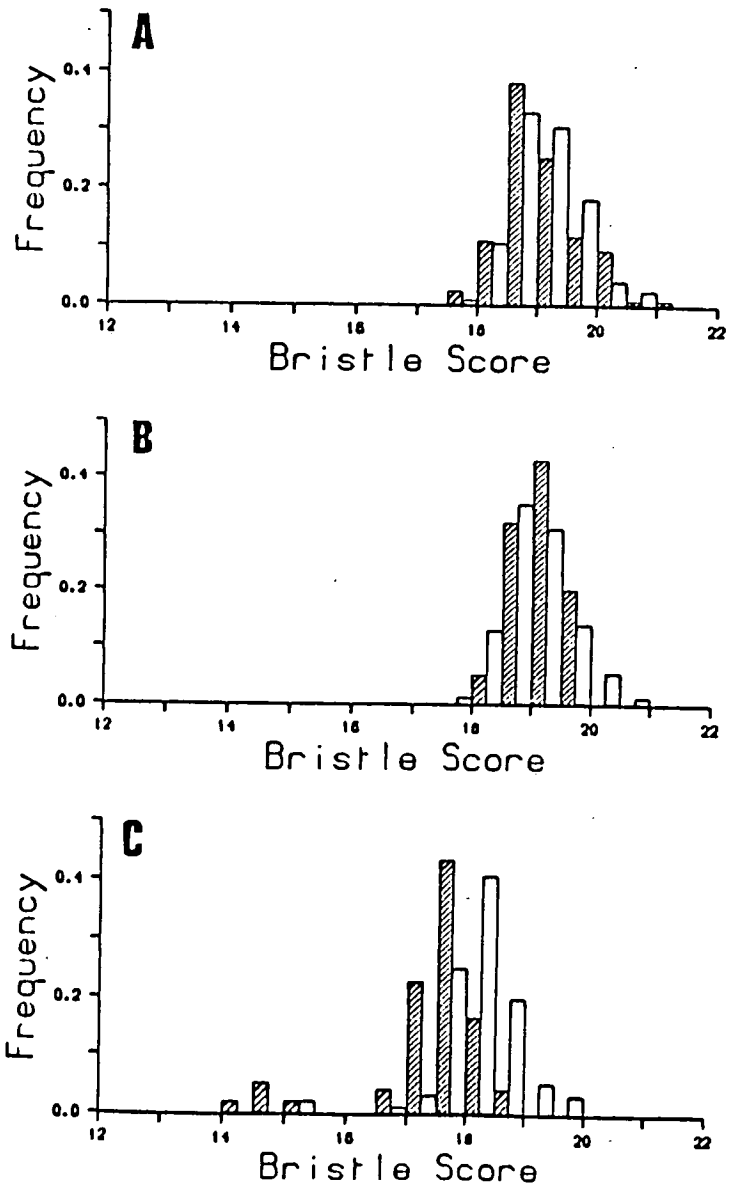

Figure 2.3 Distribution of *X* chromosome line means for sternopleural bristle score. Dysgenic (cross-hatched bars) and nondysgenic (open bars) control *M* strain *X* chromosomes are shown in panel A, dysgenic and nondysgenic *M* strain *X* chromosomes in panel B and dysgenic and nondysgenic *P* strain *X* chromosomes in panel C.

chromosome dysgenic population. One line has a mean abdominal bristle score of 11, three standard deviations below the population mean, and nine lines have mean sternopleural bristle scores over one standard deviation less than the overall mean (Figure 2.1c.). However, the mean of both bristle traits remains lower for dysgenic than nondysgenic *P* strain *X* chromosomes even if the extreme lines are excluded (differences of 0.52 abdominal and 0.51 sternopleural bristles), but these differences in mean are not statistically significant.

2.3.2 Variance Components

Estimates of between- and within-line components of variance of abdominal and sternopleural bristle score for the *X*-chromosome populations are given in Table 2.1. The expected between-line component of variance from *X* chromosomes is zero in the absence of mutation, because the *X* chromosomes are from inbred lines. However, the segregating autosomal background will contribute variance between lines; this is estimated from the dysgenic and nondysgenic control populations to be 3% of the total variance for abdominal bristles, and 10% of the total variance for sternopleural bristles. There is no clear heterozygous effect of *P*-induced autosomal polygenic mutations: between-line variance components are similar for the dysgenic and nondysgenic control populations.

Therefore *P*-induced *X*-linked polygenic mutational variation can be detected by comparing the between-line components of variance of the populations with different *X* chromosome origin and treatment to the basal level of between-line segregational variance, estimated from the

TABLE 2.1 Statistics for *M* and *P* *X* chromosome lines derived from a single dysgenic (D), nondysgenic (ND) or control (C) cross.

Cross	Source of *X*	Number of Lines	Bristle Trait	$\bar{X}$(s.e.)	$\hat{V}_b$(s.e.)	$\hat{V}_w$	Skewness(s.e.)
ND,C (6)	*M*	121	A	19.3(0.06)	0.133(0.046)	4.24	-0.05 (0.22)
			S	19.1(0.05)	0.201(0.041)	2.25	0.62 (0.22)
D, C (5)	*M*	126	A	19.3(0.05)	0.130(0.045)	3.78	-0.20 (0.22)
			S	19.1(0.06)	0.288(0.049)	2.02	0.95 (0.22)
ND (2)	*M*	94	A	19.7(0.07)	0.143(0.055)	4.33	0.27 (0.25)
			S	19.6(0.06)	0.165(0.043)	2.41	0.55 (0.25)
D (1)	*M*	R1 100	A	19.8(0.08)	0.363(0.082)	4.31	-0.63 (0.24)
			S	19.1(0.04)	0.032(0.022)	2.35	-0.08 (0.24)
		R2 148	A	19.2(0.05)	0.124(0.038)	4.00	-0.36 (0.20)
			S	19.6(0.04)	0.064(0.021)	2.21	0.15 (0.20)
ND (4)	*P*	96	A	17.5(0.06)	0.206(0.057)	3.57	-1.01 (0.25)
			S	18.1(0.07)	0.321(0.065)	2.54	-2.47 (0.25)
D (3)	*P*	97	A	16.9(0.08)	0.403(0.077)	2.68	-10.19 (0.25)
			S	17.4(0.10)	0.834(0.134)	2.09	-7.11 (0.25)

The numbers in brackets refer to the cross numbers listed in the Materials and Methods. Given are the means ($\bar{X}$), between-line ($\hat{V}_b$) and within-line ($\hat{V}_w$) components of variance, and measure of skewness (SNEDECOR and COCHRAN, 1971) for two bristle traits, abdominal (A) and sternopleural (S) bristle counts. Standard errors (s.e.) are in brackets.

R1, R2 mean Replicate 1 and Replicate 2, respectively.

average between-line variance of dysgenic and nondysgenic control populations. For the population of *M* strain *X* chromosomes passed through one nondysgenic cross, this comparison shows no significant difference in between-line variance for either bristle trait. In contrast, the first replicate of the *M* strain *X* chromosomes derived from dysgenic crosses gave a nearly 3-fold increase in the between-line component of variance of abdominal bristle score, suggesting the occurrence of dysgenesis-induced mutations affecting this trait, but a substantial reduction in the between-line sternopleural bristle variance. To determine whether this reduction in variance was a real effect or caused by sampling, a second replicate was made of 148 dysgenic *M* strain *X* chromosomes. Again, the between-line variance of sternopleural bristle score was reduced compared to the control cross; in this case the between-line variance of abdominal bristle score was not different from the control. *P* strain *X* chromosome lines derived from dysgenic crosses had between-line variances for both bristle traits elevated greater than three times the control value. *P* strain *X* chromosome lines derived from nondysgenic crosses also exhibited increased between-line components of variance, but less so than those that had undergone dysgenic crosses (a 60% increase for abdominal, and 30% increase in sternopleural bristle variance compared to the control). Inasmuch as the variance components for the bristle traits are diagnostic of the occurrence of *P*-induced bristle mutations, this analysis indicates (i) *P* strain *X* chromosomes are more mutable than *M* strain *X* chromosomes, and (ii) dysgenic crosses are more effective in generating mutations than nondysgenic crosses, but mutations arising from nondysgenic crosses involving *P* strain target chromosomes are not

negligible.

2.3.3 Mutational Variance

Mutational variances ($\hat{V}_m$) for the two quantitative traits, abdominal and sternopleural bristle number, were estimated for each of the four populations of *X* chromosomes from the difference between the observed between-line components of variance and the average control between-line variance for each trait, multiplied by a factor of five to scale the *X* chromosome estimate to the whole genome. Mutational heritabilities ($\hat{h}^2_m$) were estimated as $\hat{V}_m/(\hat{V}_m + \hat{V}_e)$, where $\hat{V}_e$, the environmental variance, was calculated from the average within-line variance of the two characters in the Harwich (*P*) and Sam (*M*) inbred strains (Table 2.3). For abdominal bristle score, $\hat{V}_e = 3.33$ and for sternopleural bristle score, $\hat{V}_e = 1.57$. The estimates are given in Table 2.2. In general, a single generation of *P* element mutagenesis generates exceptionally high levels of variation for bristle traits, leading to heritabilities of a magnitude typically observed in natural populations (Falconer, 1989). *P*-induced mutational variances are greater for *P* strain than *M* strain chromosomes, and greater for dysgenic than nondysgenic crosses. Note that whenever there is appreciable mutational heritability for a trait, the distribution of that trait is significantly negatively skewed (compare Tables 2. 1 and 2. 2), indicating the mutations predominantly reduce bristle score.

To test for the continued production of mutational variance for the two bristle traits after the initial dysgenic or nondysgenic cross, dysgenic and nondysgenic *M X* chromosomes were backcrossed to inbred

M and *P* strain females for seven generations. The first backcross generation used F_3 males from the *X* chromosome lines described above, giving nine generations over which mutations could accumulate. By the end of seven backcross generations the autosomes are expected to be over 99% isogenic with the inbred strains (Falconer, 1989a), so the only source of variation between lines is mutational variance on the *X*. The estimates of V_m from these lines are simply the between-line components of variance, scaled by a factor of five to be comparable to the whole genome, and divided by the number of generations over which mutations could arise. A conservative estimate is to assume mutations occur at an equal rate for nine generations, but it is possible that *P* mutagenesis only happens in the first few backcross generations. Since the pattern of *P* transposition is unknown in these experiments, estimates of V_m are given in Table 2.3 assuming mutagenesis for two different time periods. If *P* element mutagenesis occurs for five generations, $\hat{V}_m = \hat{V}_b$; the estimates for nine generations are in the column labelled $\hat{V}_m$.

The between-line variances for abdominal and sternopleural bristle score contributed by *M* strain *X* chromosomes derived from a single dysgenic cross were 0.112 and negative, respectively (average of replicates (1) and (2)); for nondysgenic *M* strain *X* chromosomes the estimates were not different from zero - 0.011 and negative. The between-line variance of dysgenic and nondysgenic *M* strain chromosomes backcrossed to inbred *P* or *M* strains increased compared to the initial values (Table 2.3), which is indicative of further mutagenesis during backcrossing.

Estimates of mutational heritability were calculated assuming

TABLE 2.2 Mutational variance and mutational heritability

Cross	X Chromosome	Bristle Trait	$\hat{V}_m$	$\hat{h}^2_m$
Dysgenic	*M**	A	0.561	0.144
		S	(-)	(-)
	P	A	1.356	0.289
		S	2.945	0.652
Nondysgenic	*M*	A	0.057	0.017
		S	(-)	(-)
	P	A	0.371	0.100
		S	0.380	0.195

*average of replicates (1) and (2)

Estimates of mutational variance ($\hat{V}_m$) and mutational heritability ($\hat{h}^2_m = \hat{V}_m/(\hat{V}_m + \hat{V}_e)$) generated by dysgenic or nondysgenic crosses in *P* or *M* strain backgrounds are given for abdominal (A) and sternopleural (S) bristle score. (-) indicates a negative estimate.

TABLE 2.3 Statistics for *M* strain *X* chromosomes backcrossed to inbred *P* and *M* strains.

Initial Cross	BX Strain	N	Bristle Trait	$\hat{V}_b$	$\hat{V}_w$	$\hat{V}_m$*	$\hat{h}^2_m(9)$	$\hat{h}^2_m(6)$
Dysgenic	*M*	46	A	0.215	3.29	0.134	0.039	0.061
			S	0.125	1.78	0.078	0.047	0.074
	P	39	A	0.607	3.62	0.379	0.102	0.154
			S	0.201	1.75	0.126	0.074	0.113
Nondysgenic	*M*	28	A	0.210	2.88	0.131	0.038	0.059
			S	0.093	1.39	0.058	0.036	0.056
	P	30	A	0.390	3.53	0.244	0.068	0.105
			S	0.219	1.37	0.137	0.080	0.122

Between- ($\hat{V}_b$) and within- ($\hat{V}_w$) line variance components, mutational variance ($\hat{V}_m$) and mutational heritability ($\hat{V}_m/(\hat{V}_m + \hat{V}_e)$) for abdominal (A) and sternopleural (S) bristle score are given for inbred *M* strain *X* chromosomes passed through a single dysgenic or nondysgenic cross, then backcrossed (BX) for seven generations to the inbred *P* or *M* strain. N is the number of chromosome lines of each cross. The two values of $\hat{h}^2_m$ were calculated assuming different numbers of generations of mutation accumulation (see text for explanation).

* At Generation 9, the accumulated mutational variance came from 8 generations of potential mutagenesis. So V_m = the total mutational variance of the X chromosome divided by 8; V_m was also calculated assuming 5 generations of potential mutagenesis (not shown) for the estimation of h^2_m (6).

eight ($\hat{h}^2_m(9)$) or five ($h^2_m(6)$) generations of potential mutagenesis, and using the estimates of environmental variance given above. Although the estimates assuming fewer generations of mutation accumulation were 70% larger than the most conservative estimates, both displayed the same patterns. (1) Mutational heritabilities were similar for both bristle traits. (2) The initial difference in $\hat{h}^2_m$ between *X* chromosomes subjected to one dysgenic or nondysgenic cross disappeared after seven backcross generations. (3) Mutational heritabilities from *X* chromosomes backcrossed to a *P* strain was twice that of *X* chromosomes backcrossed to an *M* strain.

2.3.4 Mutant Effects

Twelve lines have been identified with extreme bristle phenotypes on the basis of the deviation of their scores from the overall population mean. Of these, nine were observed on *P* strain *X* chromosomes after a single dysgenic cross, two on *P* strain *X* chromosomes after a single nondysgenic cross, and one on an *M* strain *X* chromosome from the nondysgenic control population at Generation 4 of maintenance. The mean sternopleural and abdominal bristle score based on a sample size of 20 individuals, deviations from the overall population mean in standard deviation units, and the Student's *t* statistic for the test of significance of the difference in mean are given in Table 2.4. Only sternopleural bristle score is affected for ten of the mutant lines, while both traits are extreme for the remaining two lines. In all cases the direction of the mutant effect is to decrease bristle score. It is interesting to note that the lines

TABLE 2.4 Bristle mutant lines

Origin	Line	Abdominal bristle			Sternopleural bristle		
		$\bar{X}$	t	SD	$\bar{X}$	t	SD
DP	98	16.8	-0.46	-0.10	15.0	-6.32*	-1.41
	103	17.1	0.31	0.07	15.2	-5.92*	-1.32
	112	16.7	-0.59	-0.13	14.9	-6.71*	-1.50
	114	17.1	0.43	0.10	14.7	-7.23*	-1.62
	126	16.7	-0.71	-0.16	14.9	-6.58*	-1.47
	146	11.5	-13.98*	-3.13	14.9	-6.71*	-1.50
	159	17.2	0.56	0.13	14.0	-8.94*	-2.00
	164	17.2	0.56	0.13	14.8	-6.84*	-1.53
	187	16.5	-1.11	-0.25	14.4	-7.89*	-1.76
NDP	108	17.0	-1.17	-0.26	15.5	-7.12*	-1.59
	149	17.5	-0.14	-0.03	15.1	-8.18*	-1.83
NDC	19	10.3	-19.44*	-4.35	11.0	-23.11*	-5.17

*$p << 0.001$

Mean ($\bar{X}$) abdominal and sternopleural bristle score, *t*-statistic, and deviation from population mean in standard deviation units (SD) for *X* chromosome lines with putative bristle mutations. These originated on *P* strain *X* chromosomes after one dysgenic cross (DP), *P* strain *X* chromosomes after one nondysgenic cross (NDP), and on a nondysgenic control (NDC) *M* strain *X* chromosome during maintenance.

with sternopleural bristle scores averaging 1.6 standard deviations below the mean were identified as mutant with reference to their base population, but otherwise their scores are well within the wild-type range. They are therefore true "polygenic" mutations. The lines with abdominal and sternopleural bristle scores greater than three standard deviations below the mean are, however, quite extreme in comparison to wild-type variation.

Assuming each line bears an independent *P*-induced bristle mutation, the polygenic mutation rate on *P X* chromosomes from one dysgenic cross is 9.3%, and from one nondysgenic cross is 2%. However, the ten lines for which only sternopleural bristle score was affected all have similar scores, and it is possible they all result from a common event involving a resident *P* element on the target *P* chromosome situated near a locus affecting bristle score. The two lines deviant for both traits have mutant phenotypes distinct from the other ten lines. Genetic and cytological mapping experiments are documented in Chapter 4 to determine allelism of these mutants, and whether they can be associated with *P* element insertions or deletions. The bristle phenotype of line number 146 (see Table 2.4) is unstable and has reverted to both more extreme and wild-type phenotypes, which suggests that this mutation at least is caused by a *P* element insertion.

2.4 DISCUSSION

The ability of the *P-M* system of hybrid dysgenesis in *Drosophila*

*melanogaster*to generate mutations affecting quantitative characters was tested by measuring the *de novo* production of variation for two bristle traits (abdominal and sternopleural bristles) between *X* chromosomes of inbred *P* and *M* strains passed through a single dysgenic (*M* ♀♀ x *P* ♂♂) or nondysgenic (*P* ♀♀ x *M* ♂♂) cross, in a common autosomal background. Dysgenic crosses are capable of inducing polygenic mutational variance, given by the mutational heritability as a proportion of the environmental variance, at levels equivalent to heritabilities of these traits from natural populations (Falconer, 1989a). This magnitude of mutagenesis is unprecedented; a single generation of exposure to *P* element movement is one hundred times more powerful than 1000 r X-rays in inducing variation for abdominal bristle count (Mackay, 1987). The sole exception is the variance of sternopleural bristle score between dysgenic *M* strain *X* chromosomes, which was less than the control between-line variance of this trait. A possible explanation, other than the obvious interpretation of sampling error as a cause of the difference, is that new mutants for this trait were lethal, and therefore not recovered. Mutational heritabilities of the bristle traits were about eight times greater when *P* strain *X* chromosomes were used as targets; this is not surprising because *P*-induced mutations on *P* chromosomes can occur by excision as well as insertion. Eight of the nine *P* strain *X* chromosomes showing extreme bristle scores had similar phenotypes, and could have been generated by excision of a *P* element near a locus affecting bristle score. Although nondysgenic crosses of *M* strain *X* chromosomes did not give rise to detectable polygenic mutations, nondysgenic crosses of *P* strain *X* chromosomes did, suggesting that the repression of *P*

transposition is not complete in this situation.

Maintenance by backcrossing to inbred *M* or *P* strains of originally isogenic *M* strain *X* chromosomes that were passed through one dysgenic or one nondysgenic cross generates further mutational variance for bristle traits. Additional mutagenesis for the case of the *M* strain backcross parent is explicable because these are dysgenic crosses. The autosomal background of the G_3 males used at the first backcross generation, whether they were derived from dysgenic or nondysgenic crosses, potentially contains intact *P* elements, and so the opportunity exists for further hybrid dysgenesis-induced *P* transposition on crossing to *M* cytotype females. However, the evolution of polygenic mutational variance among *M* strain *X* chromosomes backcrossed to inbred *P* strain females, which was twice as great as the variance induced by backcrossing to *M* strain females, is less easy to understand. These backcrosses are nondysgenic; by what mechanism could *P* elements be mobilized by nondysgenic interstrain crosses? One explanation might be that the inbred *P* strain used was altered from the original Harwich strain in terms of its ability to repress intrastrain *P* transposition, and so was unstable. Torkamanzehi *et al.* (1988) showed their subline of Harwich had high levels of intrastrain ovarian dysgenesis at 29°, suggestive of *P* element mobility within this strain. It is also possible that another hybrid dysgenesis system is operating in addition to *P-M* dysgenesis. The Harwich and Samarkand strains used are classified as *I* in the *I-R* hybrid dysgenesis system, but their status with regard to *hobo* dysgenesis (Blackman *et al.*, 1987; Yannopoulos *et al.*, 1987) is not known.

These results are comparable to those of Mackay (1986, 1987) who

demonstrated that *M* strain second chromosomes passed through initial dysgenic and nondysgenic crosses evolved similar levels of polygenic mutational variance for a number of quantitative traits after eight backcrosses to a Harwich-derived *P* strain. This effect was only detectable in comparison to variation for the traits among control *M* strain chromosomes which necessarily had a different genetic background. Torkamanzehi *et al.* (1988) and Pignatelli and Mackay (1989) found accelerated responses to artificial selection for bristle traits in lines started from nondysgenic interstrain crosses, which they interpreted in terms of new *P*-induced mutations affecting the bristle traits. The observations of Shrimpton *et al.* (1990) of equal amounts of *P* transposition in the dysgenic and nondysgenic selection lines of Mackay (1985) lend support to this interpretation. The production of *P*-induced polygenic mutations from nondysgenic as well as dysgenic crosses confounds the estimation of mutational variation unless accumulation of mutations is restricted to a single generation, but this problem should not detract from the overall conclusion that *P* elements are capable of inducing very high levels of variation for quantitative traits.

The effects of all *P*-induced mutations affecting bristle score observed on *X* chromosomes in this experiment are large, and decrease the value of the traits, giving highly negatively skewed distributions of mutant effects. The nine dysgenic *P* strain *X* chromosome mutants are responsible for all of the new variation between lines for sternopleural and abdominal bristle score; removal of these lines from the analysis drops the between-line component of variance well below the basal control value for each trait(see Table 2.5). When a few

mutations cause most of the new polygenic variation, estimates of

TABLE 2.5 Variance Components of Dysgenic and Nondysgenic Cross, *P X* Chromosome Populations Without the Mutant Lines

			Abdominal Bristle		Sternopleural Bristle	
		N	Between	Within	Between	Within
D	*P-X*	88	0.0778	2.6944	0.0761	2.1280
ND	*P-X*	94	0.1867	3.5191	0.1300	2.5723

mutational variance are expected to be noisy, and will be large when a sample contains a new mutant and negligible otherwise. This may partly explain the large variance in response among replicate selection lines started from dysgenic and nondysgenic crosses (Mackay, 1985; Torkamanzehi *et al.*, 1988; Pignatelli and Mackay, 1989). Verification of the generality of the observation that new mutations primarily decrease bristle number must await the creation of a larger sample of bristle mutations. *P* elements are capable in general of causing a spectrum of mutant effects resulting from increasing or decreasing the amount or altering the timing or tissue-specific expression of relevant gene products (Engels, 1988). *P* element mutagenesis thus offers exciting prospects for identifying and mapping quantitative trait loci in Drosophila, and understanding the genetic and molecular basis of quantitative variation.

2.5 SUMMARY

To determine the ability of the *P-M* hybrid dysgenesis system of

Drosophila melanogaster to generate mutations affecting quantitative traits, *X* chromosome lines were constructed in which replicates of isogenic *M* and *P* strain *X* chromosomes were exposed to a dysgenic cross, a nondysgenic cross, or a control cross, and recovered in common autosomal backgrounds. Mutational heritabilities of abdominal and sternopleural bristle score were in general exceptionally high – of the same magnitude as heritabilities of these traits in natural populations. *P* strain chromosomes were eight times more mutable than *M* strain chromosomes, and dysgenic crosses three times more effective than nondysgenic crosses in inducing polygenic variation. However, mutational heritabilities of the bristle traits were appreciable for *P* strain chromosomes passed through one nondysgenic cross, and for *M* strain chromosomes backcrossed for seven generations to inbred *P* strain females, a result consistent with previous observations on mutations affecting quantitative traits arising from nondysgenic crosses. The new variation resulting from one generation of mutagenesis was caused by a few lines with large effects on bristle score, and all mutations reduced bristle number.

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CHAPTER 3

EFFECTS OF *P*-ELEMENT-INDUCED POLYGENIC MUTATIONS ON BRISTLE SCORES

3.1 INTRODUCTION

To understand better the genetic basis of quantitative variation, it is necessary to know the distribution of allelic effects within and between quantitative trait loci, and the dominance and pleiotropic effects of individual polygenes. The classical genetical model for metric traits assumes continuous variation is conditioned by a large number of independent loci each with small effects. This theory has played an important role in plant and animal breeding. However, it has several major deficiencies (Robertson, 1966): it can not predict the likely limits to selection except under very simplifying conditions, and the change in reproductive fitness with selection, and it may provide a biased prediction of correlated response (Bohren *et al.*, 1966). In determining the minimum number of genes influencing a trait, most easily applied methods (Castle, 1922; Wright, 1952) are based on a model assuming equal gene effects, independent gene action, and directional distribution of allelic effects. Again, the accuracy and practical use of these methods are restricted, and further improvement requires many more parameters be estimated (Lande, 1981; Cockerham, 1986).

It has been widely recognized that the distribution of effects of

mutant genes on quantitative characters may be highly leptokurtic (Robertson, 1967, 1968; Mackay, 1989). Thoday (1967, 1973) and Thoday and Thompson (1976) located a total of nine major sternopleural bristle effects situated on all three major chromosomes of *Drosophila melanogaster* accounting for accelerated response observed during selection. Shrimpton and Robertson (1988a, b) found a minimum of 17 "factors" to be responsible for the difference in score of 24 sternopleural bristles. The largest sternopleural effect detected was nearly $3\sigma_p$, and other effects ranged down to the limit of detection of $0.5\sigma_p$, where σ_p is the phenotypic standard deviation. With a leptokurtic distribution of mutant effects, genes with a large effect are few in number, but will contribute greatly to mutational variance, and the large number of genes with small effects contribute less to mutational variance.

It is has been observed (reviewed by Simmons and Crow, 1977) that there is a negative correlation between homozygous effect and degree of dominance for viability mutations of *Drosophila melanogaster*. The effect of new mutations on heterozygous fitness is severe, with a heterozygous fitness reduction equal to the homozygous viability effect. However, less information is known of the dominance properties of quantitative morphological traits, such as bristle number and body size. Pleiotropic effects of major morphological mutants on other traits, including fitness, are well known (for examples in Drosophila, see Lindsley and Grell, 1968). However, there are few data on the pleiotropic effects of new mutations with more subtle effects on quantitative traits. More experimental data are required to answer these questions.

In the experiment discussed in Chapter 2, it was shown that *P*-

induced mutational quantitative variation for sternopleural and abdominal bristle scores mainly resulted from 11 mutant *X* chromosomes. The goal of this chapter is to investigate the additive, dominance and pleiotropic effects of these mutant chromosomes.

3.2 MATERIALS AND METHODS

Materials

Drosophila Stocks: All flies were reared at 20-22°C on 10 ml agar-yeast-glucose medium in shell vials. The gene markers and chromosomes used are described in Lindsley and Grell (1968).

1) *FM4,*y^{31d} sc^{8} *dm B; SAMARKAND* (SAM). The *FM4 X* chromosome has been substituted into the inbred SAM background (see Chapter 2) by 21 generations of backcrossing.

2) *FM4,*y^{31d} sc^{8} *dm B; T(2,3)ap*^{Xa}, SAM. Its *X* chromosomes are identical to those of strain 1 above. The second and third chromosomes are balanced by the dominantly marked translocation, and the rest of genome is derived from inbred SAM. The above two stocks were kindly supplied by Dr. Trudy F.C. Mackay.

3) Tested Mutant lines. Eight hybrid dysgenesis-induced *X* chromosome mutant lines were tested (see Chapter 2, Table 2.4). Three of the original mutant lines were unfortunately lost due to poor fitness, and one reverted to wildtype, during maintenance. Males of mutant lines DP(98), DP(103), DP(126), DP(159), DP(164), NDP(108) and NDP(149) have low sternopleural bristle scores of about 15, and wildtype abdominal bristle scores. DP(146) and NDC(19) males have

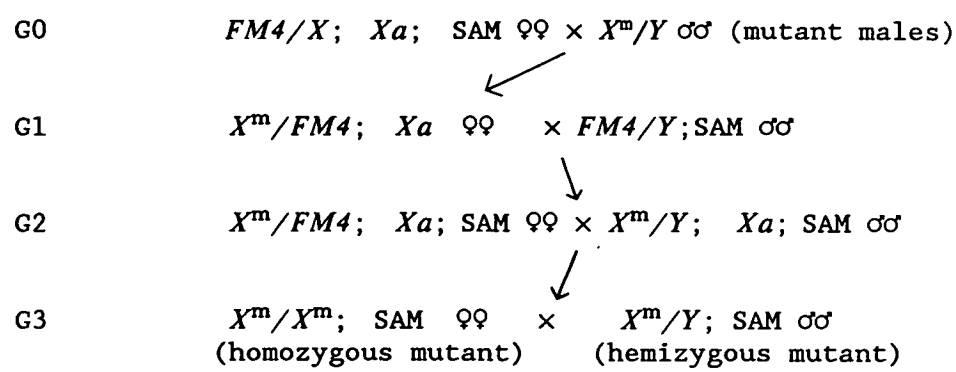


Figure 3.1: Crossing scheme to make the mutant *X* chromosomes homozygous in a SAM inbred background.

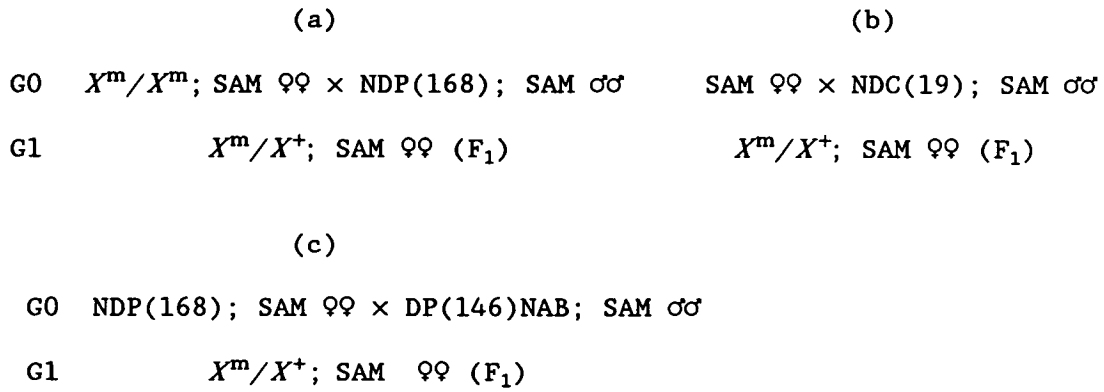


Figure 3.2: Crossing schemes to produce females heterozygous for mutant and wildtype *X* chromosomes in a SAM inbred background.

extremely low abdominal and sternopleural bristle scores of 11.5 and 15.5; and 10.7 and 13.0; respectively. One mutant line, DP(146)NAB, which spontaneously arose from mutant line DP(146) during maintenance, has nearly no abdominal bristle, with a mean of 1.5, but a similar sternopleural bristle score (15.7) to DP(146). These mutant lines were kept by backcrossing to *C(1)DX* SAM (see Chapter 2) for more than 10 generations, then the *X* chromosomes were made homozygous and the autosomes isogenic with inbred SAM. The method is illustrated in Fig.3.1. Mutant males were crossed to *FM4;Xa;* SAM females, then F_1 females heterozygous for *FM4* and *Xa* were backcrossed to *FM4*; SAM males. In the following generation females heterozygous for *FM4* and *Xa* were mated to *Xa* males with the mutant phenotype. Finally homozygous mutant females and hemizygous mutant males were mated *inter se*. The fitness of homozygous mutant females varies. NDC(19) females are completely sterile, while those of DP(103) are partially sterile. DP(146)NAB females are lethal, but females of the original mutant line DP(146) are viable and fertile with occasional reversion of the mutant phenotype to wildtype. Other mutant lines are reasonably fit and stable.

4) Tester Strain 1, NDP(168); SAM. This is a wildtype strain, which was chosen at random from 20 lines that originated from the nondysgenic cross, *P* strain *X* chromosome population (see Chapter 2), and which retained the wildtype phenotype for 10 generations. The *X* chromosome was made homozygous and the autosomes substituted with inbred SAM chromosomes by the procedure described above. This strain was used as a tester for the mutant lines for which it is co-isogenic (all but NDC(19)).

5) Tester Strain 2, inbred *SAMARKAND (SAM)*. This was used as

a tester for NDC(19), as it is isogenic with this line. Its origin is described in Chapter 2. It was full-sib inbred for 36 generations before use.

Means and standard errors of abdominal and sternopleural bristle scores of the two testers are given in Table 3.1.

The above strains were used to assess the effects of the mutant chromosomes in hemizygous males, and in homozygous and heterozygous females in the SAM inbred autosome background.

Methods

With the exception of mutant lines NDC(19) and DP(146)NAB, 10 homozygous mutant females were crossed with 10 males of tester strain 1, NDP(168); SAM (see Fig. 3.2a), with two replicates for each cross. Sternopleural and abdominal bristle scores of the homozygous mutant females were recorded before the mating. DP(146)NAB females are homozygous lethal, so 10 males were crossed with 10 females of tester strain 1 (see Fig.3.2c) with two replicates to produce heterozygous females.

Since homozygous females of NDC(19) are sterile, two replicate crosses of 10 males of this mutant line with 10 females of tester strain 2, SAM (see Fig.3.2b), were made to produce heterozygous mutant females. Two days later these mutant males from each replicate were re-mated to two replicates of 10 *FM4;*SAM females, then their heterozygous *FM4* F_1 female progeny were backcrossed to 10 mutant males to derive homozygous mutant females. The two bristle traits were recorded

for 10 homozygous females from each replicate.

In the F_1 generation, 40 heterozygous females from each replicate of each mutant line were measured for the traits of interest. Abdominal and sternopleural bristle scores of 15-20 males of each mutant line were also determined.

3.3 RESULTS

Means and standard errors (pooled over two replicates) of *X* chromosome mutants in the SAM inbred background are given for homozygous and heterozygous females, and hemizygous males, in Table 3.2. Because mutant DP(146)NAB is lethal in females, the mean male bristle scores of this line were used in the following estimations.

3.3.1 Effects of Mutant Chromosomes in Females

The homozygous and heterozygous effects of the mutant *X* chromosomes were estimated in the following manner. If M^{++} is the mean of the homozygous tester (NDP(168); SAM or SAM) females, and M^{mm} the mean of homozygous mutant females, the additive effect, *a*, of the mutant chromosome is $(M^{mm} - M^{++})/2$ (Falconer, 1989a). If M^{m+} is the mean of heterozygous females, the heterozygous effect, *d*, of the mutant chromosome is $M^{m+} - (M^{mm} + M^{++})/2$ (Falconer, 1989a). Values of *a*, *d*, their ratio (the degree of dominance, *d/a*), and *a* scaled by the phenotypic standard deviation (σ_p) of the tester females (NDP(168); SAM or SAM) are given in Table 3.3. The standard errors (s.e.) of

TABLE 3.1 Means (±Standard Errors) of Tester Strains

		Abdominal Bristles	Sternopleural Bristles
NDP(168) SAM	♀	20.40 ± 0.380	19.65 ± 0.365
	♂	17.30 ± 0.273	19.28 ± 0.253
SAM	♀	22.35 ± 0.357	21.05 ± 0.256
	♂	19.30 ± 0.298	20.75 ± 0.189

TABLE 3.2 Means (±Standard Errors) of Homozygous and Heterozygous Females and Hemizygous Males for Mutant X Chromosomes

	Abdominal Bristle Scores			Sternopleural Bristle Scores		
	Male	Female		Male	Female	
		Homozygote	Heterozygote		Homozygote	Heterozygote
DP(98)	17.36±0.411	20.55±0.398	19.92±0.289	15.42±0.229	15.00±0.343	18.95±0.273
DP(103)	17.08±0.571	18.39±0.593	19.92±0.291	15.40±0.289	15.05±0.322	18.24±0.236**
DP(126)	18.10±0.362	19.55±0.722	19.83±0.280	15.55±0.223	15.10±0.386	18.33±0.244*
DP(146)	11.15±0.314	11.80±0.337	17.93±0.261	15.16±0.377	15.75±0.321	18.04±0.221
DP(159)	17.82±0.685	21.45±0.629	20.01±0.285	15.40±0.221	15.30±0.431	19.04±0.229
DP(164)	17.31±0.463	21.00±0.527	19.96±0.287	15.38±0.340	15.65±0.264	18.80±0.245
NDP(108)	17.80±0.395	21.35±0.530	19.99±0.302	15.30±0.242	15.50±0.342	18.63±0.232
NDP(149)	17.18±0.325	20.05±0.591	20.43±0.258	15.35±0.209	15.65±0.366	19.38±0.262**
NDC(19)	9.57±0.369	10.75±0.481	22.54±0.324	12.14±0.261	13.55±0.530	18.16±0.225
DP(146) NAB	1.60±0.536		17.90±0.319	15.50±0.423		18.19±0.284

**: P<<0.01, *: P<<0.05.

homozygous (a) and heterozygous (d) effects were estimated by: s.e.(a)=$\sqrt{[\sigma^2(mm)/n_1 + \sigma^2(++)/n_2]}$, and s.e.($d$)=$\sqrt{\{\sigma^2(m+)/n_3 + (n_1+n_2)/(4n_1n_2)\cdot[\sigma^2(mm) + \sigma^2(++)]\}}$, where n_1, n_2, n_3, $\sigma^2(mm)$, $\sigma^2(++)$ and $\sigma^2(m+)$ are the sample size and pooled variance over replicates of homozygous mutant females (mm), homozygous tester females (++) and heterozygous females (m+), respectively.

Sternopleural Bristle Scores: Except for line NDC(19), all mutant chromosomes have a similar average additive effect of -2.14 bristles. Mutant line NDC(19) has the largest additive effect of -3.75 bristles. The dominance effects vary among the mutant chromosomes. DP(98), DP(103), DP(126), DP(159), DP(164), NDP(108) and NDP(149) have similar sternopleural bristle scores of about 15, but their heterozygous effects range from 0.89 to 1.74. These values are not significantly different from each other (t-test, $P<0.05$). Interestingly, *an* F -test indicated that mean sternopleural bristle scores among heterozygous females of seven mutant chromosomes are significantly different (see Table 3.2). In particular, the means of mutant lines DP(103) and DP(126) are significantly smaller, and the mean of line NDP(149) is substantially higher, than the rest. However, differences among means of homozygous females of these lines for the same trait are not significant. This suggests that these mutant lines may have different dominance properties, although the actual estimates of d are not significantly different. The ratio of d/a, the degree of dominance, consistently indicates the effects of the mutants on this trait are partially recessive, with an average degree of dominance for these seven mutant chromosomes of -0.596. In contrast, mutant lines DP(146), DP(146)NAB and NDC(19) have more nearly additive effect (the average

TABLE 3.3 Estimates of Additive and Dominance Effects (±Standard error) of the X Chromosome Polygenic Mutations on Bristle Scores in Females

	Abdominal Bristles				Sternopleural Bristles			
	-a	d	-d/a	$-a/\sigma_p$	-a	d	-d/a	$-a/\sigma_p$
DP(98)					2.33±0.250	1.63±0.467	0.70	1.43
DP(103)					2.30±0.243	0.89±0.436	0.39	1.41
DP(126)					2.28±0.265	0.95±0.464	0.42	1.39
DP(146)	4.30±0.248	1.83±0.468	0.43	2.53	1.95±0.243	0.34±0.433	0.24	1.20
DP(159)					2.18±0.282	1.56±0.471	0.72	1.33
DP(164)					2.00±0.225	1.15±0.430	0.58	1.23
NDP(108)					2.08±0.250	1.05±0.445	0.51	1.27
NDP(149)					2.00±0.258	1.74±0.468	0.87	1.27
NDC(19)	5.80±0.302	5.99±0.537	1.02	3.63	3.75±0.322	0.86±0.453	0.23	3.14
DP(146) NAB	9.40±0.300	3.45±0.564	0.37	5.53	2.08±0.279	0.62±0.498	0.30	1.27

a: additive effect on bristle number.
d: dominance effect.
-d/a: degree of dominance.
σ_p: the phenotypic standard deviation of the wildtype tester females.

d/a for these mutant lines is -0.26). The degree of dominance averaged over all mutant chromosomes is -0.494.

Abdominal Bristle Scores: Only mutant lines DP(146), DP(146)NAB and NDC(19) were examined for this trait, as the abdominal bristle phenotypes of the other mutant chromosomes did not differ significantly from the wildtype. Additive and dominance effects of lines DP(146) and DP(146)NAB are -4.3 and 1.83, -9.4 and 3.45, respectively; they are partly recessive. However, line NDC(19), with additive and dominance effects of -5.8 and 5.99, is completely recessive to the wildtype.

3.3.2 Effects of Mutant Chromosomes in Males

The effects of the mutant chromosome in males (α) were calculated by subtracting the mean sternopleural or abdominal bristle score of the wildtype tester males (NDP(168); SAM or SAM) from the mean bristle score of the mutant males. Estimates of α and of α/σ_p are given in Table 3.5, where σ_p is the phenotypic standard deviation of the wildtype tester males. The effects of NDC(19) on abdominal and sternopleural bristle scores are -9.73 and -8.61, respectively. Mutant line DP(146) also has a large effect of -6.19 on abdominal bristle scores, and a lesser effect of -4.12 on sternopleural bristle scores, which is similar to the effect of the other eight mutants on sternopleural bristle score. Mutant line DP(146)NAB has an exceptionally large effect (-15.7) on abdominal bristle score. All other mutant *X* chromosomes had similar effects on sternopleural bristles only, with an average α of 3.88.

TABLE 3.4 Estimates of Effects (± Standard Errors) of *X* Chromosome Polygenic Mutations on Bristle Scores in Males

	Abdominal Bristles			Sternopleural Bristles		
	$-\alpha$	$-\alpha/\sigma_p$	$-2a$	$-\alpha$	$-\alpha/\sigma_p$	$-2a$
DP(98)				3.86±0.341	2.41	4.65
DP(103)				3.88±0.384	2.42	4.60
DP(126)				3.73±0.338	2.33	3.90
DP(146)	6.19±0.416	3.58	8.6	4.12±0.454	2.57	4.35
DP(159)				3.88±0.336	2.42	4.35
DP(164)				3.90±0.424	2.44	4.00
NDP(108)				3.98±0.350	2.48	4.55
NDP(149)				3.93±0.328	2.45	4.00
NDC(19)	9.73±0.474	5.16	11.60	8.61±0.350	7.23	7.50
DP(146) NAB	15.70±0.473	9.09		3.78±0.388	2.36	

α: mutant effect on bristle number in males.
2a: mutant effect on bristle number in females (see Table 3.3), where a is additive effect.
σ_p: the phenotypic standard deviation of the wildtype tester males.

Baker and Belote (1983) found that Drosophila genes on the single *X* chromosome of males are regulated to produce the same amount of product as the two female copies, and this phenomenon of dosage compensation in males operates at the transcriptional level. As a consequence, the effect on bristle traits estimated from mutant males (α) is expected to equal twice the additive effects ($2a$) estimated from the homozygous mutant females. These values are listed for comparison in Table 3.4. Most of the mutant lines have similar values of α and $2a$. The exceptions are DP(146) and NDC(19). The mean abdominal bristle score of homozygous DP(146) females is greater than hemizygous males, and the mean sternopleural bristle score of homozygous NDC(19) females is less than hemizygous males.

3.4 DISCUSSION

3.4.1 The Range of Mutant Effects

The range of additive effects among these ten mutant lines for both bristle traits is 2 to 9.5 bristles (1.2-5.5 σ_p) in females and 3.7 to 15.7 bristles (2.3-9.1 σ_p) in males. These estimates are similar to the values obtained previously (see Chapter 2) by expressing the means of each mutant line in terms of their standard deviation from the mean of the population from which they derived. The effects of nine mutant lines for sternopleural bristle score are close to 1.3 σ_p, well within the wildtype range. However, the effects of mutant line NDC(19) for both bristle traits, and mutant lines DP(146) and DP(146)NAB for

abdominal bristle numbers, are extreme compared with the wildtype, and exceed the largest effect found by Shrimpton and Robertson (1988a,b) for sternopleural bristle scores. It is possible the extreme effects of these *X* chromosome lines are caused by more than one mutation. This appears to be the case for mutant line DP(146)NAB, a dramatically unstable secondary mutation derived from mutant line DP(146). The attempt to map this mutation failed, partly because of the instability and partly because there may be double mutations in the *X* chromosome. However, the other nine mutants initially arose within a single generation, including the extreme mutant NDC(19), and were mapped to single loci (see Chapter 4). Overall the results lend support for the conjecture that the distribution of effects of polygenic mutations are highly variable, and possibly leptokurtic among loci. (Robertson, 1967, 1968; Mackay, 1989).

3.4.2 Dominance Effects

The reduction of sternopleural bristle scores in mutant lines DP(146), DP(146)NAB and NDC(19) are mainly through their additive effects. The overall degree of dominance of all mutants suggests they are partially recessive, with mutant line NDC(19) appearing completely recessive in its effect on abdominal bristle score. It has been widely observed that most visible mutations are recessive. The work of Kacser and Burns (1981) provides a theoretical explanation for this observation. Enzymes participating in biochemical pathways *in vivo* are kinetically linked to other enzymes through their substrate and products. This interaction of all enzymes comprises a system, called

flux, which modifies the effects of enzyme variation on the phenotype. A decrease in the activity of a particular enzyme may be compensated by an increase in pool size of its substrate or products with a little or no observable change in flow rate of flux. A reduction to 50% activity in the heterozygote, a widespread feature observed for many mutants, is therefore not expected to be detected in the phenotype. The mutant would therefore be considered "recessive". The prevailing occurrence of recessive mutants is thus seen to be the unpreventable consequence of the kinetic structure of enzyme networks. The dominance properties of the bristle phenotype mutations is roughly in accord with this hypothesis, as they are recessive (NDC(19)) or partly recessive, although Drosophila bristle phenotype may not be relevant to the enzyme "flux". However, although no dominant *X* chromosome mutant was found in this experiment, a dominant third chromosome mutant (later identified as an allele of *Delta*), which increased abdominal bristle scores in heterozygotes by 10 bristles, was detected during maintenance of the *X* chromosome lines. Other dominant mutations affecting quantitative traits have been observed (e.g., *Hw, H and D* affecting Drosophila bristle score (Lindsley and Grell, 1968); *Hg* affecting postweaning growth rate in mice (Bradford and Famula, 1984)). They are not accounted for by the theory of Kacser and Burns (1981), which considers the effects of mutations which decrease the gene product. However, the *Dl* mutation results from inactivating a suppressor gene (Taghert *et al.*, 1984; Doe and Goodman, 1985). The dominance properties of new mutations may well depend on the wildtype function of the inactivated gene, and also on whether the mutation causes an increase or decrease of gene product at a particular locus.

Simmons and Crow (see review, 1977) pointed out that there is an association between homozygous effects and degree of dominance for viability mutations in Drosophila; mutations with extreme homozygous effects are more recessive. In contrast, the homozygous effects and degree of dominance of the mutants affecting bristle score show little correlation (see Table 3.3). Although the mutant lines described here only represent a small population and therefore cannot be used to infer overall associations, two particular mutant lines display different patterns of heterozygous effects. NDC(19) has a large effect in homozygotes and is completely recessive for abdominal bristle scores, but also has a large homozygous effect on sternopleural bristle score and is only partly recessive for this trait. DP(146) has a large homozygous effect on abdominal bristle score, and a moderate homozygous effect on sternopleural bristle score, but is partly recessive for both traits. The sex-linkage of these mutations may account for the discrepancy between this result and Simmons and Crow's observations.

3.4.3 Pleiotropic Effects

Most loci simultaneously affect more than one trait (for examples in Drosophila, see Lindsley and Grell, 1968). Three of the ten mutants have associated effects on both bristle traits and viability. The direction of effects of each mutant line on all traits is towards decreasing the bristle number and viability (see Chapter 4), but the individual associations of abdominal and sternopleural bristle scores are obviously quite variable (4.3 and 1.95 bristles of DP(146), 5.8 and 3.75 bristles of NDC(19), and 9.4 and 2.75 bristles of DP(146)NAB).

For mutant lines DP(146) and NDC(19), these appear to be true pleiotropic effects of single mutations, since in both cases the effects have not been separated by recombination (see Chapter 4).

It is a common observation that most mutations have similar dominance relationships for all the characters they affect. Interestingly, the degree of dominance of each of the three pleiotropic mutants on both characters are different, particularly mutant NDC(19), for which the abdominal bristle effect is completely recessive, but the sternopleural bristle effect is only partially recessive. This observation can be interpreted by the model established by Keightley and Kacser (1987). If enzymes act 'linearly', that is in the absence of saturation and feedback inhibition or other nonlinearity, all fluxes and pools conditioned by a mutant gene have identical dominance relationships to different characters. The presence of nonlinearity, however, leads to diversity in dominance relationships between different characters. These mutants genes may fit the latter category; the enzymes they specify may act differentially on abdominal and sternopleural bristles, by sustaining different substrate concentrations and/or different activation or induction of some of the enzymes, leading to different degrees of dominance of the two characters.

3.5 SUMMARY

The range of additive effects among the ten polygenic mutants of the *X* chromosome of *Drosophila melanogaster* for both abdominal and

sternopleural bristle scores are 2 to 9.5 bristles (1.2-5.5σ_p) in females, and 3.7 to 15.7 bristles (2.3-9.1σ_p) in males. The similar effects of nine mutant chromosomes (the exception is NDC(19)) on sternopleural bristle scores are close to 1.3σ_p, well within the wildtype range. The effects of line NDC(19) for both bristle traits, and lines DP(146) and DP(146)NAB for abdominal bristle scores, are extreme compared with the wildtype, and exceed the largest effect found by Shrimpton and Robertson (1988a, b) for sternopleural bristle score. This result supports the hypothesis that the distribution of effects of mutant genes on quantitative characters may be highly leptokurtic.

Although the reduction of sternopleural bristle scores of mutant lines DP(146), DP(146)NAB and NDC(19) are mainly additive, the overall degree of dominance of all mutant *X* chromosomes suggests they are partially recessive; line NDC(19) appears completely recessive in its effect on abdominal bristle score. This is in agreement with the observation that most visible mutations are recessive.

CHAPTER 4

MAPPING AND CHARACTERIZATION OF *P*-ELEMENT-INDUCED POLYGENIC MUTATIONS IN *Drosophila Melanogaster*

4.1 INTRODUCTION

In the experiment described in Chapter 2, I derived twelve *X* chromosome lines with mutations affecting abdominal and sternopleural bristle scores, using dysgenic and nondysgenic interstrain crosses between inbred Harwich (*P* strain) and inbred Samarkand (*M* strain) in which *P* elements were mobilized. These mutants were potentially caused by insertions or excisions of *P* elements, or chromosome rearrangements. One important advantage of TE mutagenesis is that if the TE-induced mutations are insertions, then the wildtype locus can be cloned by the transposon-tagging method (Bingham *et al.*, 1981). However, before cloning is possible, the precise location of the mutant effects must be mapped and the map position(s) must be correlated with the presence of a *P* element insertion. This chapter documents further mapping and characterization of these mutant *X* chromosome lines, and is divided into four parts. In the first part the mutations were mapped genetically relative to morphological markers using an approach similar to that of Thoday (1961) and Shrimpton and Robertson (1988a,b). The second part was an operational test of allelism of these mutants. In the third part deficiency mapping was performed to localize chromosomal

positions of mutants. Finally, the technique of *in situ* hybridization was utilized to pinpoint the site of insertion of *P* elements that are associated with the mutant loci.

4.2.1 MAPPING OF POLYGENIC MUTATIONS

Materials

Drosophila Stocks: The gene markers and chromosomes used are described in Lindsley and Grell (1968). All flies were reared on 10 ml G-Y medium at about 20-22°C in shell vials.

1) The tested mutant lines: They are ten *X* chromosome mutants (the other three mutant lines were lost during maintenance) for abdominal and sternopleural bristle scores derived from the *P* element mutagenesis experiment (see Chapter 3, Table 3.2). Mutant lines DP(98), DP(103), DP(126), DP(159), DP(164), NDP(108) and NDP(149) have low sternopleural bristle scores of about 15, and wildtype abdominal bristle scores. Mutant lines DP(146) and NDC(19) have extremely low abdominal bristle scores of 11.5 and 10.8, respectively, and sternopleural bristle scores of 15.5 and 13.0, respectively. The autosomes of these mutant lines are nearly isogenic with inbred SAM as a result of backcrossing to *C(1)DX* SAM for over 10 generations. This experiment was initiated before the one described in Chapter 3, so the autosomes of these mutant lines might not be absolutely identical to those described in Chapter 3.

2) Wildtype strain NDP(168); SAM: This wildtype stock is isogenic

to all mutant lines except NDP(19), as it was maintained by backcrossing to *C(1)DX* SAM. Males of this stock have wildtype abdominal and sternopleural bristle scores of about 17.5 and 19 (also see Table 3.1, Chapter 3), respectively.

3) *X* Chromosome Marker A : This stock, supplied by Bowling Green Drosophila Stock Center, carries the multiple recessive markers: *y* ct^6 t^2 *v f car*, and has abdominal and sternopleural bristle scores of 17.8 and 20.8 in males.

4) *X* Chromosome Marker B: This stock was derived from *X* chromosome marker A by eliminating the *y* marker and has the genotype : ct^6 t^2 *v f car*. Approximately 75% of its autosomes are common to SAM since it was backcrossed to *C(1)DX* SAM for two generations before use.

5) Inbred SAM and *C(1)DX* SAM: The origins of these strains are described in Chapter 2.

1) Mutant Lines DP(98), DP(103), DP(126), DP(159), DP(164), NDP(149) and NDP(108)

As these seven mutant lines have similar sternopleural bristle scores, it is possible they derived from imprecise or precise excision of a *P* element inserted near a locus affecting bristle score on the parent *P* chromosome. Therefore, these mutant lines have been treated as one group. The first step is to determine in which region of the *X* chromosome each of these mutants is situated.

The crossing scheme is shown in Figure 4.1. 4 vials with 8

mutant males and 8 homozygous marker A females (*y* ct^6 t^2 *v f car*) were established for each mutant line. Then 10 F_1 females were backcrossed to 10 males of the marker A stock per vial, with 20 replicate vials for each mutant. Due to the difficulty in scoring the t^2 phenotype reliably when flies have the *y* phenotype, the t^2 phenotype was temporarily ignored and only four types of recombinant males were collected. The *X* chromosome of each mutant line was thus divided into four separate "sections" flanked by adjacent marker loci, e.g., section 1 is the chromosomal segment between the *y* and *ct* loci, and is 20 cM in length (section 2 is 13 cM, section 3 is 23.7 cM, and section 4 is 7.8 cM).

Sternopleural bristle scores of 10-20 male recombinant flies containing different sections of each mutant *X* chromosome were recorded. In addition, a control was conducted in the same way using the wildtype stock NDP(168). Discrepancies among the means of the four sections of the *X* chromosome within each mutant line, and between the mutant and wildtype means within the same section of the *X* chromosome, would indicate in which *X* chromosome section the mutant genes are present.

The mean sternopleural bristle scores of each chromosomal section are given in Table 4.1. Comparison of means among each section within each mutant line, and between mutants and the control within the same section, indicates that all seven mutant genes appear to be within section 1 or section 2 (e.g., in the *y-v* interval). Further classification of + + *v f car* recombinants into two groups (see Table 4.2, + + t^2 *v f car* and + + + *v f car*) on the basis of the t^2 phenotype suggests these mutants are most likely to be in section 2 (within ct^6-t^2).

The mean sternopleural bristle scores of section 2 recombinants

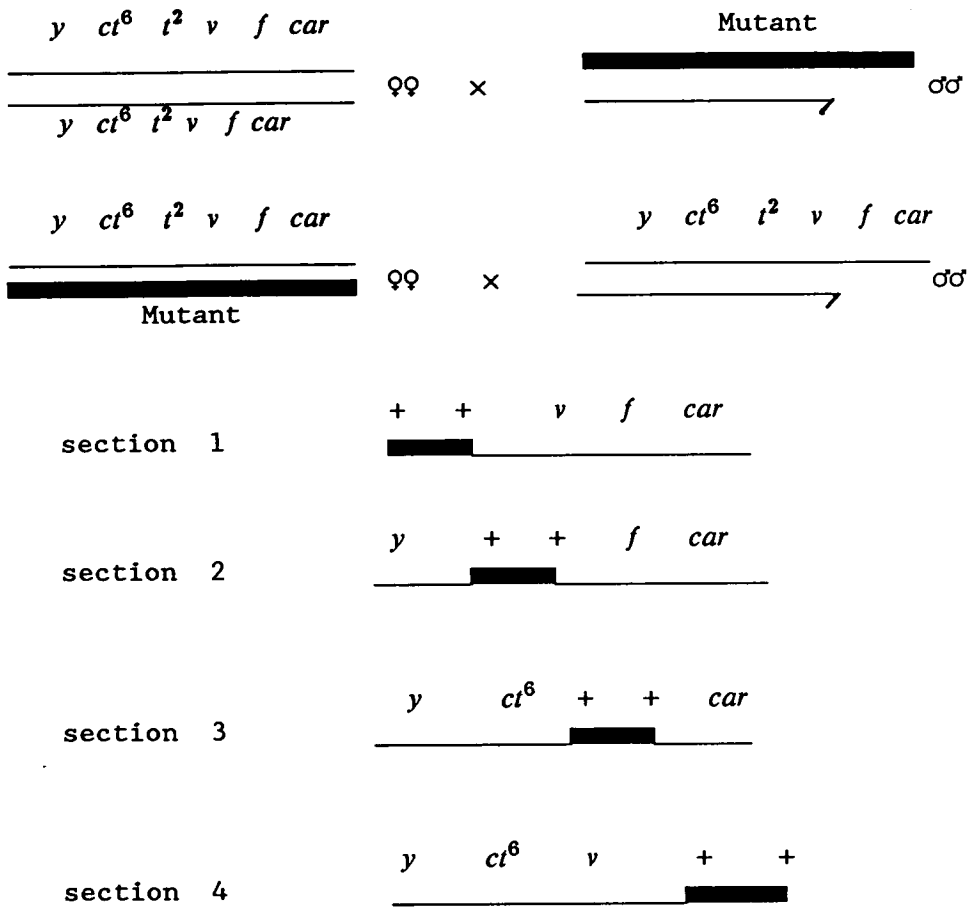


Figure 4.1 The *X* chromosome marker A (*y ct*^{6} *t*^{2} *v f car*) females were mated to mutant males of each *X* chromosome line, then F_1 females were backcrossed to marker A males. Each mutant *X* chromosome was divided into four sections (ignoring the *t*^{2} marker), e.g. section 1 is the chromosomal segment between the *y* and *ct* loci, section 2 between *ct* and *v*, section 3 between *v* and *f*, and section 4 between *f* and *car*.

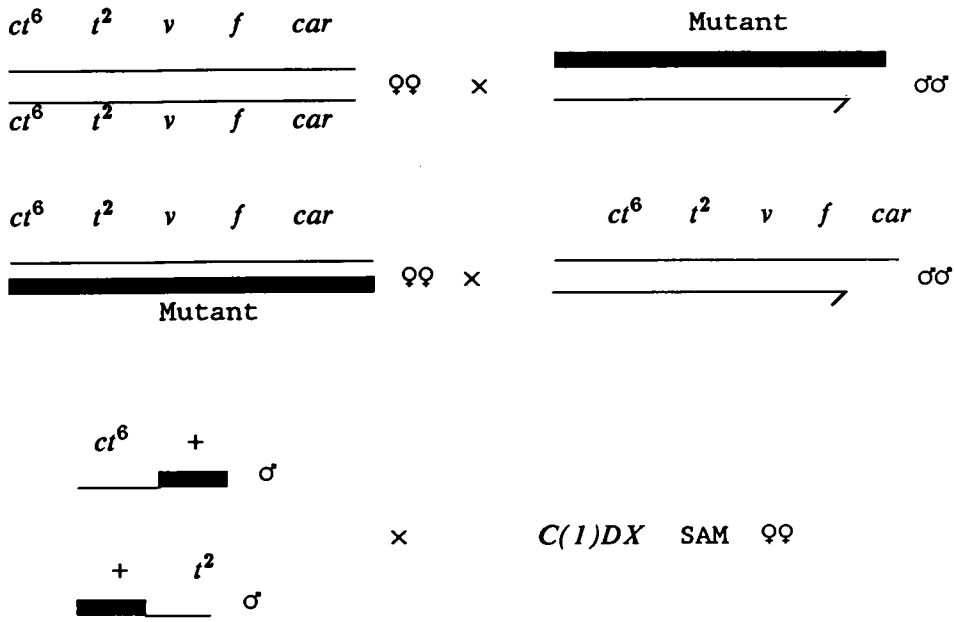


Figure 4.2 The *X* chromosome marker B (ct^6 t^2 *v f car*) females were crossed with mutant males of each *X* chromosome line, then F_1 females mated to marker males, and recombinant ct^6 + and + t^2 males were collected and crossed individually with *C(1)DX* SAM females to establish recombinant lines for each mutant.

TABLE 4.1 Means (±standard error) of Recombinant Males of the *X* Chromosomal Sections of Mutants for Sternopleural Bristle Scores

	Recombinant Classes			
Line	+ + *v f car*	*y* + + *f car*	*y ct*^{6} + + *car*	*y ct*^{6} *v* + +
DP(98)	18.00±0.451 (25)	17.25±0.446 (12)	19.88±0.875 (8)	20.11±0.301 (18)
DP(103)	17.53±0.289 (38)	16.88±0.580 (8)	20.00±1.023 (7)	20.67±0.518 (18)
DP(126)	19.00±0.562 (19)	18.35±0.428 (17)	19.70±0.360 (7)	20.35±0.386 (20)
DP(159)	17.55±0.400 (19)	17.00±0.392 (13)	20.30±0.559 (10)	20.36±0.326 (27)
DP(164)	18.11±0.307 (37)	17.52±0.328 (21)	20.38±0.680 (8)	19.79±0.379 (19)
NDP(149)	18.00±0.417 (23)	17.00±0.370 (20)	19.20±0.916 (5)	20.22±0.475 (18)
NDP(108)	17.94±0.503 (17)	16.60±0.371 (10)	20.20±0.860 (5)	21.10±0.318 (18)
NDP(168) Wildtype	19.44±0.428 (16)	22.25±0.552 (12)	20.17±0.543 (6)	19.56±0.250 (16)

The numbers in the brackets are numbers of recombinant males scored.

TABLE 4.2 Means (±standard error) of Recombinant Males After Classification of + + *v f car* Recombinants

	n	+ + t^2 *v f car*	n	+ + + *v f car*
DP(98)	15	19.20 ± 0.490	10	16.70 ± 0.474
DP(103)	21	18.33 ± 0.340	17	16.53 ± 0.375
DP(126)	12	20.08 ± 0.621	7	17.14 ± 0.670
DP(159)	10	18.40 ± 0.476	9	16.11 ± 0.389
DP(164)	24	18.29 ± 0.428	13	17.77 ± 0.378
NDP(149)	12	19.25 ± 0.604	9	17.67 ± 0.624
NDP(108)	9	19.56 ± 0.377	8	16.13 ± 0.398

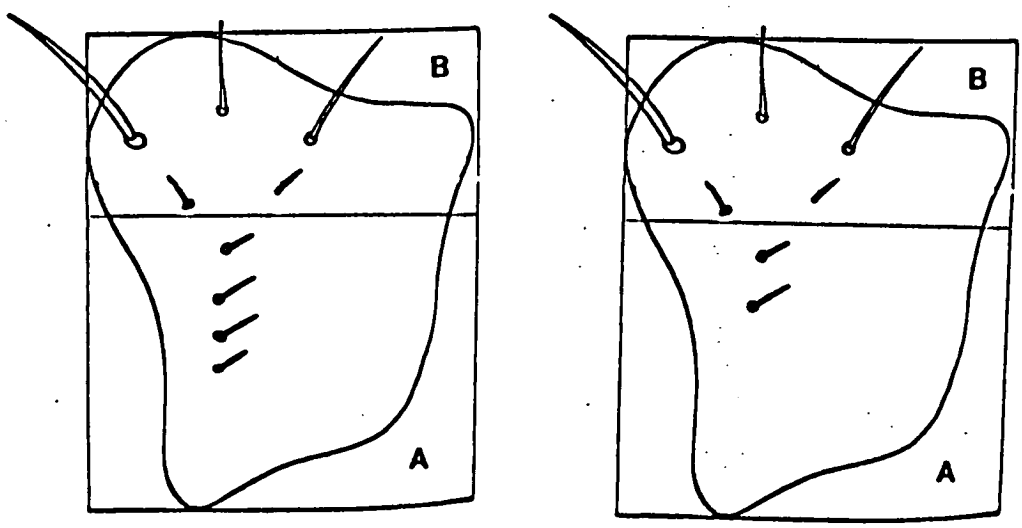
n is the number of recombinant males scored

(*y + + f car*) in Table 4.1 and *+ + + v f car* recombinants in Table 4.2 are higher than 15, the overall mean of the seven mutant lines for this trait, because these flies have different autosomes from those of the tested mutant lines.

To localize more precisely the position of each mutant gene, a second mapping experiment was conducted. The crossing scheme is given in Figure 4.2. Mutant males were mated to females of the *X* chromosome marker stock B (*ct*^{6} *t*^{2} *v f car*), then F_1 females were backcrossed to marker males. Fifty *ct*^{6} + and + *t*^{2} recombinant males each were collected for each mutant *X* chromosome in the following generation. It is possible in this generation to classify *ct*^{6} + and + *t*^{2} recombinant males into wildtype and mutant flies according to individual sternopleural bristle scores. However, this could bias the estimate of map distance because scoring only one individual to determine whether it is wildtype or mutant could result in a large sampling error. To derive more precise estimates, each single recombinant male was crossed to 5 *C(1)DX* SAM females, and 10 male offspring were measured for sternopleural bristle scores. Therefore, approximately 50 *ct*^{6}+ and 50 + *t*^{2} recombinant *X* chromosome lines were established for each mutant line. The means of each line are listed in Tables 4.3-7, except DP(103) and DP(98) which will be discussed later. Based on the line means, the *ct*^{6} + and + *t*^{2} recombinant *X* chromosomes were aligned in the order of minimum to maximum and grouped into wildtype and mutant categories. In the case where means of recombinant lines are well divided, such as DP(164), the recombinant lines were split into two categories directly. However, when the means of recombinant lines were less clear-cut, they were grouped into wildtype and mutants in following manner: 1) Recombinant

lines were divided into two groups such that the variance within each group is minimal. In Table 4.3-7, $S_{\bar{X}}$ is the least standard deviation among means of the recombinant lines within mutant or wildtype classes, after they were split into wildtype and mutant. 2) The sternopleurite of flies on each side can be divided into two regions A and B (see Fig 4.3). The reduction of sternopleural bristle scores in these mutant individuals is due to one or two bristles missing in region A on each side of sternopleurites. This provides an alternative method to distinguish mutant recombinants from wildtype recombinants.

The variance between lines within mutant and wildtype classes may be due to effects of mixed autosomes from mutant lines, markers and *C(1)DX*SAM strains, and sampling error.



Wildtype Mutant

Figure 4.3 Sternopleurites on each side of *Drosophila melanogaster* are divided into region A and B. The left is wildtype, the right is mutant.

TABLE 4.3 Means of Recombinant Lines in Males between ct^6-t^2 of Line DP(126) for Sternopleural Bristle Scores (ST)

ct^6 +				+ t^2			
Mutant *		Wildtype *		Mutant		Wildtype	
Line	ST	Line	ST	Line	ST	Line	ST
47	13.5	29	17.1	47	14.7	25	17.4
1	13.9	33	17.3	33	14.8	18	17.4
24	13.9	48	17.6	1	14.9	6	17.7
18	13.9	45	17.6	45	15.0	12	17.7
39	14.0	17	17.8	27	15.1	41	17.7
37	14.4	11	17.9	44	15.2	4	17.7
6	14.4	49	18.0	43	15.3	17	17.7
4	14.6	26	18.1	21	15.4	7	17.7
14	14.7	2	18.1	10	15.5	30	17.8
35	14.7	28	18.1	3	15.6	29	17.8
20	14.8	9	18.2	31	15.6	8	17.9
38	14.8	19	18.9	19	15.7	42	17.9
43	14.8	12	19.3	34	15.8	9	17.9
8	14.8	30	19.4	16	15.8	28	18.0
13	14.8	44	19.6	40	16.0	2	18.0
27	14.8	15	20.1	11	16.1	13	18.0
41	14.9	50	20.5	35	16.3	46	18.0
31	14.9					32	18.1
7	15.0	$\bar{X}$=18.45		$\bar{X}$=15.4		36	18.1
34	15.0	$S_{\bar{X}}$=0.9708		$S_{\bar{X}}$=0.4538		22	18.3
25	15.1	n=17		n=17		38	18.3
5	15.1					37	18.7
10	15.2					48	18.8
42	15.3					14	18.8
23	15.5					26	18.8
40	15.6					15	18.8
3	15.7					24	18.8
46	15.8					5	19.0
32	15.9					20	19.3
36	16.1					23	19.5
21	16.2					39	20.9
22	16.3						
16	16.4					$\bar{X}$=18.27	
						$S_{\bar{X}}$=0.7240	
$\bar{X}$=14.99						n=31	
$S_{\bar{X}}$=0.7228							
n=33							

* Mutant and Wildtype refer to sternopleural bristle phenotypes in Fig. 4.3. This also applies to Tables 4.4 - 4.7.

TABLE 4.4 Means of Recombinant Lines in Males between ct^6-t^2 of Line DP(159) for Sternopleural Bristle Scores(ST)

ct^6 +				+ t^2			
Mutant		Wildtype		Mutant		Wildtype	
Line	ST	Line	ST	Line	ST	Line	ST
10	13.6	29	16.9	21	14.9	11	18.3
19	13.8	21	17.4	23	15.2	15	18.4
4	13.9	6	17.4	18	15.3	4	18.7
5	13.9	1	17.6	1	15.9	25	18.7
33	13.9	17	17.6	20	16.3	17	18.8
7	14.1	18	17.6	19	16.3	27	18.9
15	14.1	8	17.9	10	16.7	22	19.0
9	14.2	2	18.1			12	19.0
22	14.2	31	18.2	$\bar{X}$=15.8		14	19.4
25	14.5	32	18.3	$S_{\bar{X}}$=0.6256		26	19.4
23	14.5	24	18.4	n=7		6	19.5
34	14.6	20	18.5			3	19.6
37	14.6	30	18.5			2	19.7
27	14.6	28	18.5			8	20.0
36	14.8	11	18.7			24	20.1
16	14.9	26	18.9			13	20.3
13	14.9	12	19.9			9	20.3
38	15.0					7	20.7
14	15.0	$\bar{X}$=18.14				16	20.8
35	15.9	$S_{\bar{X}}$=0.7074				5	21.0
3	16.0	n=17					
						$\bar{X}$=19.53	
						$S_{\bar{X}}$=0.8096	
$\bar{X}$=14.50						n=20	
$S_{\bar{X}}$=0.6289							
n=21							

TABLE 4.5 Means of Recombinant Lines in Males between ct^6-t^2 of Line DP(164) for Sternopleural Bristle Scores(ST)

ct^6 +				+ t^2			
Mutant		Wildtype		Mutant		Wildtype	
Line	ST	Line	ST	Line	ST	Line	ST
4	13.7	45	18.3	9	14.5	36	18.4
11	14.0	43	18.5	10	14.6	27	18.4
22	14.1	31	18.5	2	15.1	21	18.5
13	14.2	41	18.5	6	15.1	36	18.6
20	14.3	36	18.8	8	15.4	15	18.6
18	14.4	39	18.9	4	15.6	25	18.7
25	14.5	37	18.9	3	15.8	22	18.8
8	14.5	33	19.2	1	15.8	29	18.9
2	14.5	30	19.3	11	15.8	20	18.9
6	14.5	42	20.0	7	15.9	35	19.0
23	14.7	38	20.0	5	16.1	24	19.1
19	14.7	34	20.0			37	19.1
9	14.7	29	20.1	$\bar{X}$=15.43		12	19.2
21	14.9	40	20.5	$S_{\bar{X}}$=0.5368		13	19.2
7	14.9	35	20.5	n=11		17	19.3
17	14.9	32	20.7			14	19.4
5	14.9	44	20.9			28	19.4
16	15.1					16	19.5
26	15.1	$\bar{X}$=19.51				23	19.5
27	15.1	$S_{\bar{X}}$=0.8764				31	19.5
15	15.2	n=17				32	19.7
3	15.3					18	19.8
24	15.4					19	19.9
12	15.4					34	20.0
10	15.6					33	20.1
1	15.6					30	20.4
28	15.7						
14	15.7					$\bar{X}$=19.23	
						$S_{\bar{X}}$= 0.5467	
$\bar{X}$=14.8						n=26	
$S_{\bar{X}}$=0.5371							
n=28							

TABLE 4.6 Means of Recombinant Lines in Males Between ct^6-t^2 of Line NDP(108) for Sternopleural Bristle Scores(ST)

ct^6 +				+ t^2			
Mutant		Wildtype		Mutant		Wildtype	
Line	ST	Line	ST	Line	ST	Line	ST
32	13.5	30	17.1	17	14.3	3	17.2
16	13.8	4	17.5	24	15.0	40	17.5
35	14.4	38	17.6	19	15.0	38	17.6
29	14.5	28	17.8	32	15.2	37	17.6
34	14.6	27	17.9	39	15.4	28	17.9
36	14.6	12	18.0	10	15.5	29	18.0
14	14.6	24	18.4	8	15.5	7	18.0
23	14.6	2	18.5	9	15.5	6	18.2
20	14.7	6	18.7	33	15.6	4	18.3
5	14.7	31	18.8	2	15.7	41	18.3
11	14.7	33	19.3	22	15.8	35	18.3
8	14.8	19	19.3	34	15.9	21	18.5
21	14.9	10	19.8	20	16.0	16	18.6
22	15.0	26	20.2	43	16.0	14	18.6
1	15.0	9	20.7	27	16.2	15	18.8
37	15.0					30	18.8
3	15.2	$\bar{X}$=18.64		$\bar{X}$=15.50		18	18.8
25	15.3	$S_{\bar{X}}$=1.0146		$S_{\bar{X}}$=0.4718		13	18.9
13	15.3	n=15		n=15		36	19.0
15	15.6					26	19.3
17	15.6					42	19.3
7	15.7					1	19.4
18	15.8					23	19.5
						12	19.6
$\bar{X}$=14.86						25	19.6
$S_{\bar{X}}$=0.5456						5	19.7
n=23						11	20.2
						31	20.5
						$\bar{X}$=18.72	
						$S_{\bar{X}}$=0.8186	
						n=28	

TABLE 4.7 Means of Recombinant Lines in Males Between ct^6-t^2 of Line NDP(149) for Sternopleural Bristle Scores(ST)

ct^6 +				+ t^2			
Mutant		Wildtype		Mutant		Wildtype	
Line	ST	Line	ST	Line	ST	Line	ST
41	13.0	14	17.0	34	15.0	37	17.8
31	13.4	4	17.6	17	15.2	12	18.2
19	13.5	8	17.8	38	15.3	13	18.3
7	13.7	16	18.0	9	15.4	19	18.5
38	14.0	2	18.1	16	15.5	18	18.6
10	14.2	29	18.2	8	15.8	30	18.6
13	14.2	11	18.3	32	16.0	14	18.8
42	14.4	35	18.3	5	16.0	20	18.8
1	14.4	36	18.4	29	16.2	36	19.0
37	14.5	28	18.4	33	16.3	15	19.1
21	14.6	9	18.4	6	16.4	24	19.1
23	14.7	34	18.6	11	16.7	28	19.2
26	14.9	30	19.0			10	19.3
24	14.9	6	19.1	$\bar{X}$=15.81		4	19.3
25	14.9	39	19.4	$S_{\bar{X}}$=0.5026		3	19.3
22	15.0	17	19.7	n=12		35	19.3
27	15.1	18	19.8			31	19.4
32	15.3	12	19.8			22	19.5
3	15.4	5	19.8			23	19.5
20	15.4					7	19.6
15	15.5	$\bar{X}$=18.63				2	19.7
33	15.6	$S_{\bar{X}}$=0.7894				27	19.8
40	16.0	n=19				26	20.0
						1	20.2
$\bar{X}$=14.64						21	20.5
$S_{\bar{X}}$=0.7476						25	20.8
n=23							
						$\bar{X}$=19.24	
						$S_{\bar{X}}$=0.6820	
						n=26	

TABLE 4.8 Means of Recombinant Lines in Males between Mutant Gene(m) of DP(98) and t^2 for Sternopleural Bristle Scores(ST)

m*		t^2 +					
Line	ST	Line	ST				
25	15.0	74	17.6	73	19.0	92	19.8
9	15.1	27	18.0	54	19.0	67	19.8
4	15.4	63	18.1	66	19.1	31	19.8
13	15.5	55	18.2	61	19.1	96	19.9
12	15.6	77	18.3	78	19.1	42	20.0
2	15.8	47	18.3	34	19.1	59	20.0
24	15.8	91	18.3	40	19.2	60	20.1
20	15.9	49	18.3	45	19.2	46	20.2
5	15.9	79	18.5	86	19.2	50	20.4
7	16.0	62	18.5	82	19.3	29	20.4
11	16.0	94	18.5	88	19.3	53	20.5
6	16.0	69	18.5	71	19.3	58	20.6
21	16.1	95	18.6	48	19.3	97	21.0
23	16.1	38	18.6	70	19.3		
16	16.2	83	18.6	43	19.4	$\bar{X}$=19.18	
14	16.3	32	18.7	30	19.4	$S_{\bar{X}}$=0.6658	
18	16.3	68	18.7	51	19.4	n=73	
22	16.3	41	18.7	84	19.4		
8	16.4	90	18.7	80	19.4	m	+
10	16.4	39	18.7	52	19.5		
19	16.4	81	18.8	56	19.5	100	15.0
3	16.7	26	18.8	72	19.6	99	15.4
17	16.7	98	18.8	57	19.6		
15	16.8	44	18.9	64	19.6	+	t^2
1	16.8	93	18.9	85	19.7		
		36	18.9	89	19.7	102	19.0
$\bar{X}$=16.06		26	18.9	35	19.7	101	19.2
$S_{\bar{X}}$=0.4856		33	19.0	28	19.7		
n=25		65	19.0	37	19.7		

m: phenotype of mutant line DP(98).

The number of wildtype and mutant lines among ct^6 + and + t^2 recombinants are listed in Table 4.9 for each *X* chromosome line, except DP(103) and DP(98). The symmetrical proportions of the wildtype and mutants within the ct^6 + and + t^2 recombinant classes confirms the above deduction that all five mutant genes are present within the ct^6-t^2 interval.

The map distance (p) of each mutant gene within section 1 was estimated by the method of maximum likelihood (Mather, 1938) as $p= (n_1 + n_2)/n$, with standard error $s.e.(p)=\sqrt{(p(1-p)/n)}$, assuming double crossovers between ct^6 and t^2 are negligible. n_1 is the number of mutant lines among recombinant lines of ct^6 + , n_2 is the number of wildtype lines among recombinant lines of + t^2, and n is the total number of ct^6 + and + t^2 recombinant lines . Since the map distance between *ct* and *t* is 7.5 cM, the approximate map distance of each mutant gene to the *ct* locus in centimorgans is estimated as 7.5p. These values are presented in Table 4.9. The map distances of the five mutant genes (DP(126), DP(159), DP(164), NDP(108) and NDP(149) are not significantly different, and so may be mutations at the same locus (χ^2 from the homogeneity test with d.f.= 4 is 0.88, $0.90< p< 0.95$; Snedecor and Cochran, 1971). This possibility was investigated by a direct test of allelism (see section 4.2.2).

Because only a few (out of 50) ct^6 + and + t^2 recombinant lines of mutant DP(98) survived, it is impossible to measure the map distance of this mutant gene based on the means of recombinant lines. Fortunately, the number of mutants and wildtypes among ct^6 + and + t^2 recombinant males were recorded by directly scoring ct^6 + and + t^2 individuals prior to mating them to *C(1)DX* SAM females. These values

are presented also in Table 4.9. The map position is about 1 cM different from that of the other five mutants. To confirm this result, a second mapping test was conducted by mating + t^2 recombinants of DP(98) to wildtype females of the SAM stock. Then F_1 females were backcrossed to + t^2 DP(98) males, 100 male offspring were collected at random and mated individually with 5 *C(1)DX* SAM females to give 100 recombinant lines. Sternopleural bristle scores were counted on 10 males from each line. According to the means and t^2 phenotype of each line (see Table 4.8), the 100 lines were classified into four groups: + +, m +, m t^2, + t^2 (m here means mutant DP(98) phenotype), from which the map distance was estimated. It is in close agreement with that obtained by scoring recombinant individuals.

The mutant gene of DP(103) was initially indicated to be in the interval of ct^6-t^2 (see above). However, later mapping to localize the precise position yielded no recombinant between ct^6 and t^2. *In situ* hybridization (for detail of method, see Appendix 1) analysis of F_1 female larvae of mutant line DP(103) males and SAM females reveals the presence of an inversion with breakpoints at approximately 5B and 8D on the *X* chromosome of mutant line DP(103) (see Fig. 4.4). Interestingly, the breakpoints of 5B and 8D happen to be the previous sites of *P* element insertions. Evidently the inversion occurred in the four generation interval between the two mapping experiments.

2) Mutant Lines NDC(19), DP(146) and DP(146)NAB

These three mutant lines can be distinguished from the wildtype

and the other six mutant lines by their exceptionally low bristle scores. The locations of the mutations were mapped using a similar procedure to that described above (see Figure 4.1), where mutant males were crossed with the marker strain A (*y ct*^{6} *t*^{2} *v f car*) females, then F_1 females were backcrossed to the marker males.

Almost every recombinant male progeny of genotype + *ct*^{6} *t*^{2} *v f car* for mutant chromosomes NDC(19) and DP(146) expressed the mutant

Figure 4.4 DP(103) *X* chromosome containing *P* elements (short arrows) against SAM *X* chromosome in a heterozygote female larva. The long sharp arrows depict the regions of inversion with breakpoints at approximately 5B and 8D.

TABLE 4.9 The Estimated Map Distances of Six Mutants with Sternopleural Bristle Scores of About 15

	ct^6 +		+ *t*^{2}		n	p (s.e.)	Map distance to *ct* (cM)
	Mutant n_1	Wild	Mutant	Wild n_2			
DP(98)	24	26	27	24	101	0.475(0.049)	3.56
DP(126)	33	17	17	32	99	0.657(0.048)	4.92
DP(159)	21	17	7	20	65	0.615(0.060)	4.62
DP(164)	28	17	11	26	82	0.657(0.052)	4.94
NDP(108)	23	15	18	28	84	0.607(0.053)	4.58
NDP(149)	22	18	13	28	81	0.617(0.054)	4.63

TABLE 4.11 The Estimated Map Distances of Two Mutants With Extremely Low Abdominal and Sternopleural Bristle Scores

	y +		+ *ct*^{6}		n	p (s.e.)	Map distance to *y* (cM)
	Mutant n_1	Wild	Mutant	Wild n_2			
DP(146)	1	340	242	4	587	0.008(0.004)	0.17
NDC(19)	0	515	72	0	587	0.000(0.000)	0.00

TABLE 4.10 Bristle Scores of Male Recombinants + ct^6 t^2 v f car of Mutants DP(146) and NDC(19)

♂	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	$\bar{X}$
DP(146)																					
AB	8	9	10	9	9	11	10	8	9	7	10	10	11	9	10	11	11	9	11	11	9.65
ST	15	16	16	15	16	16	15	16	16	15	17	16	17	15	16	16	16	15	15	15	15.70
NDC(19)																					
AB	10	8	11	9	11	10	10	9	10	12	11	11	11	12	9	11	8	12	10	12	10.35
ST	14	12	12	10	13	10	12	10	15	13	13	15	15	13	13	14	13	13	13	14	12.85

phenotype. The values of 20 recombinant males of each mutant line for both bristle traits are listed in Table 4.10. The mutant genes carried by these two mutants are apparently within the y-ct^6 region. To estimate the map distance of these mutant loci, two types of recombinant male flies, + ct^6 and y+ , were collected and classified directly as mutant and wildtype. The phenotypes of these mutants are sharply different from the wildtype, so no error is introduced by scoring individuals. The method of estimating map distance is as described above.

The map distances for the mutant genes given in Table 4.11 suggests that they are close to the y locus at the tip of the X chromosome. The NDC(19) gene is tightly linked to the y locus; no crossovers separating it from y occurred in 587 chromosomes. Although the map distance between the two mutant genes is not significantly different, they are distinct from each other phenotypically. Mutant NDC(19) has lower sternopleural bristle scores than mutant DP(146), and is sterile when homozygous, whereas the DP(146) homozygote is viable. These mutants were tested for allelism directly (see below).

In the other hand, the disproportionate number of + ct^6 and y + recombinants is due to low viability of each mutant. Based on these data, and given both mutant genes are closely linked to the y locus, their relative viabilities (v) can be crudely estimated (Mackay, 1986) as v = 72/515 = 0.140 for NDC(19), and v = 0.712 for DP(146). Moreover, although both mutant lines have sternopleural bristle scores similar to those of the six mutants mapped above, the bristle scores of + ct^6 t^2 v f *car* recombinants (see Table 4.10) implies that both mutant lines result from single gene mutations with pleiotropic effects on two bristle traits, rather than two different mutant genes, one of which is

similar to those of the six mutants described above.

The attempt to map mutant gene(s) carried by mutant line DP(146)NAB failed partly because it is very unstable, and partly it might contain double mutations on the *X* chromosome. The data given in Table 4.12 suggest one of the two mutant genes is DP(146), and the other may be located in the chromosomal section from *f* to the centromere.

4.2.2 TEST OF ALLELISM

Materials and Methods

The purpose of this test was to investigate allelism of the eight mutants mapped above. The basic principle of this test is well known. These mutants are recessive to the wildtype (Chapter 3), so if F_1 females heterozygous for two mutant chromosomes are wildtype, the mutations are at different loci. If the mean of F_1 females is more extreme or similar to that of parental females, both mutants are at the same locus.

All crosses were maintained in 10 ml G-Y medium at 25°C. Mutants DP(98), DP(126), DP(159), DP(164), NDP(108) and NDC(149) map closely together, and were tested as Group A, whereas mutant DP(146) was examined with mutant NDC(19) as Group B. The crossing scheme is an incomplete diallelic mating (Tables 4.13 and 4.14).

Each cross comprises two replicates with 10 males and 10 females

TABLE 4.12 Male Recombinants of Mutant DP(146)NAB with Markers

Recombinant	Bristle Score Phenotype		
	DP(146)NAB	DP(146)	Wildtype
+ *ct*^{2} *v* *f* *car*	3	35	18
+ + *v* *f* *car*	1	36	15
+ + + *f* *car*	0	82	30
+ + + + *car*	0	4	0
y + + *f* *car*	0	1	16
y *ct*^{2} + + *car*	0	0	6
y *ct*^{2} *v* + +	0	0	36
+ *ct*^{2} *v* *f* +	4	1	0
+ + + + +	149	49	38

TABLE 4.13 Means (+ standard error) for Sternopleural Bristle Scores of F1 Females from Possible Crosses among Group A Mutants in the Complementation Test

	DP(98)	DP(126)	NDP(149)	NDP(108)	DP(159)	DP(164)
DP(98)	15.63±0.220	15.25±0.270	15.63*±0.206	15.68±0.299	15.63±0.204	15.08*±0.223
DP(126)		14.83±0.279	15.10±0.280	15.20±0.296	15.10±0.259	14.63*±0.277
NDP(149)			15.13±0.257	15.28±0.218	15.33±0.255	15.30±0.247
NDP(108)				15.58±0.256	15.18±0.215	15.30±0.235
DP(159)					15.23±0.258	14.83*±0.215
DP(164)						15.43±0.230

* significantly different from one of the parents'
$P \leq 0.05$

TABLE 4.14 Means (+ standard error) for Bristle Scores of F_1 Females in Complementation Test of Group B Mutants

		DP(146)	NDC(19)	DP(164)	NDP(168)
DP(146)	AB	7.48±0.409	16.53±0.267	16.05±0.339	16.98±0.357
	ST	15.70±0.424	15.53±0.240	16.63±0.296	16.68±0.296
NDC(19)	AB		7.80±0.267		
	ST		12.35±0.274		
DP(164)	AB				18.82±0.347
	ST				17.61±0.364

P≤0.05
NDP(168): A wildtype strain.
AB: Abdominal bristle scores.
ST: Sternopleural bristle scores.

per replicate. Sternopleural bristle scores of 20 F_1 females were recorded for each replicate in Group A, while sternopleural and abdominal bristle scores were measured in Group B with the same sample size.

Results

The pooled means and standard errors of bristle scores of females of each mutant line and F_1 progeny of the crosses among mutant lines are presented in Tables 4.13 and 4.14.

In Table 4.13, the means and standard errors of sternopleural bristle scores of females of each mutant in Group A are listed diagonally, while the off-diagonals are those of F_1 female progeny of crosses between mutants. The results of *t*- test show that the means of F_1 females for most pair matings among the mutant lines are either significantly lower (more extreme) than or similar to their parental means. The exception is the F_1 females of the cross between NDP(149) and DP(98), the mean of which is significantly higher than that of NDP(149). Clearly these six mutant genes are at the same locus, in agreement with the result of physical mapping. However, the significant diversity (see Chapter 3) among means of their heterozygous females with wildtype, but similarity in means of their homozygous females, may suggest these mutants are different alleles at the same locus.

Since the two bristle scores of mutant NDC(19) males are almost

identical to those of its homozygous females (see Chapter 3) which are sterile, these two traits were recorded only from male flies of NDC(19) in this experiment. According to Table 4.14, the means of F_1 females between NDC(19) and DP(146) for abdominal bristle scores are substantially and significantly higher than those of their parental females. It appears that these lines carry mutations at separate loci. The mean for sternopleural bristle scores of F_1 females resembles that of DP(146), but is significantly higher than that of NDC(19). This presents an interesting case of two mutant loci with pleiotropic effects on two traits which complement on the basis of one trait and partially complement on the other.

To check that Group A mutants are different from Group B mutants, one mutant line (DP(164)) from Group A was paired with mutant lines DP(146) from Group B (see Table 4.14). The F_1 females appear wildtype (are significantly different from the parent scores) for both traits, showing both mutant genes are at separate loci. However, the mean of F_1 females is significantly lower than those of F_1 females from a true wildtype line and mutant line DP(164). This may indicate that there is epistasis between mutant line DP(146) and mutant lines of Group A.

4.2.3 DEFICIENCY MAPPING

Materials and Methods

Drosophila stocks: All deficiency stocks were provided by Indiana University Drosophila Stock Centre. They are listed in Table 4.15. All

flies were raised on 10ml cornmeal-agar-molasses medium at 25°C in shell vials.

Methods: (i) Mutant lines DP(146) and NDC(19). Since deficiency stocks 763 and 722 have deficiencies only in heterozygous females, 10 mutant males were mated with 10 females of each of these deficiency stocks. Strains 901, 936 and 3370 contain deficiencies only in their males, therefore 10 homozygous females of DP(146) were crossed to 10 of these deficiency males, while 10 mutant NDC(19) males were mated with

Table 4.15 Deficiency Stocks

Stock	Genotype	Breakpoints
763	*Dp(1;f) 107/In(1)sc^{8},Df(1)sc^{8}, w^{8}*	01A01; 01B02
722	*Df(1) 260-1, y/FM4*	01A01; 01B04-06
901	*Df(1)svr, spl ras^{2} fw/y^{2}y 67g/C(1)DX, y f*	01A01; 01B09-10
936	*Df(1)64c18, g sd/w^{+}y/C(1)DX,y w f*	02E01; 03C02
950	*Df(1)RA2/FM7c*	07D10; 08A04-05
951	*Df(1)KA14/FM7c*	07F01-02; 08C06
3370	*Df(1)su(s)83,y cho ras v/y^{2} scY/C(1)DX,y f*	01B10; 01D06-E01

TABLE 4.16 Means (±standard errors) of F_1 Heterozygous Females of Mutant and Control *X* Chromosomes and Deficiency Chromosomes for Bristle Traits of Interest

F1 Heterozygote	Sample Size n	Means ± standard error	
		Abdominal	Sternopleural
DP(146) × 936	16	17.44±0.483	17.50±0.250
DP(146) × 763	17	20.70±0.731	18.00±0.374
DP(146) × 722	19	14.63±0.392	16.53±0.370
DP(146) × 901	16	10.81±0.400	13.94±0.544
DP(146) × 3370	12	19.33±0.512	18.83±0.534
NDC(19) × 901	lethal		
NDC(19) × 722	lethal		
NDC(19) × 763	18	20.39±0.479	16.83±0.519
DP(126) × 950	52		16.60±0.135
DP(126) × 951	25		13.92±0.208
Control × 936	17	17.18±0.335	17.94±0.277
Control × 763	15	21.20±0.499	20.20±0.449
Control × 722	17	16.88±0.283	17.00±0.332
Control × 901	27	15.89±0.299	15.38±0.278
Control × 3370	12	19.92±0.609	21.25±0.579
Control × 950	31		17.71±0.246
Control × 951	26		17.77±0.250

Control is NDP(168); SAM.

10 heterozygous females between SAM *FM4* females and these deficiency stock males. F_1 females without *FM4* were collected to measure abdominal and sternopleural bristle scores.

(ii) Mutant lines DP(98), DP(126), DP(159), DP(164), NDP(108) and NDP(149). As these are apparently allelic, only one of the mutants, DP(126) was used in the test. 10 mutant males were crossed to stocks 950 and 951 females, respectively, and F_1 females without the *FM7c* phenotype were collected for counting sternopleural bristle scores.

For each deficiency stock a control cross was conducted in the same manner as the test cross by using the wildtype strain NDP(168);SAM.

Results

Means and standard errors of F_1 heterozygous females for bristle traits of interest are given in Table 4.16. When NDC(19) males are crossed with stocks 901 and 722, F_1 heterozygous females are completely lethal. This is puzzling, since NDC(19) homozygous females are completely sterile, but viable F_1 heterozygous females of this mutant and 763 are wildtype, suggesting that NDC(19) gene is within chromosomal bands of 1B02;1B04-06. Compared to the control, F_1 heterozygous females of line DP(146) and stocks 936, 763 and 3370 are apparently wildtype, and stock 722 are nearly wildtype, but F_1 females of this line and strain 901 display the mutant bristle phenotype.

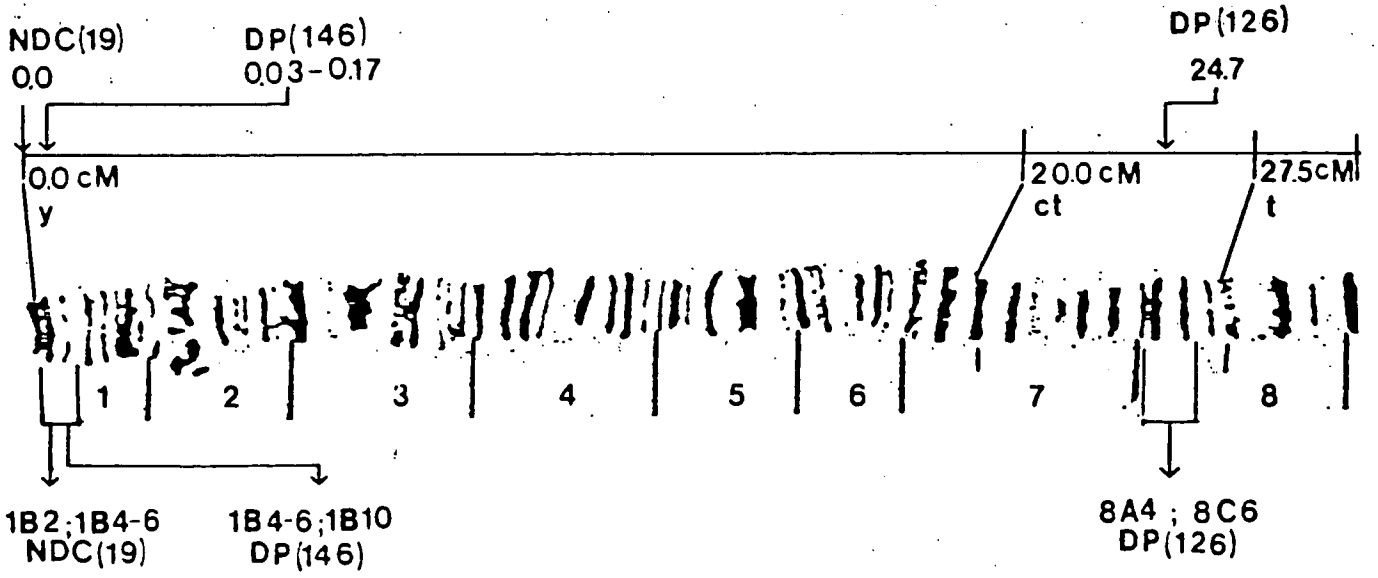


Figure 4.5 Physical mapping (above) and deficiency mapping (below) results of X chromosome mutant lines. Mutant genes DP(98), DP(159), DP(164), NDP(108) and NDP(149) are at the same position as DP(126).

This suggests mutation in DP(146) is in the region of 1B04-06;1B10, but not at 1B10, because F_1 heterozygote of this mutant and 3370 are wildtype.

The data from line DP(126) tested with stocks 950 and 951 show mutation in this line is apparently within the interval of 8A04;8C06. Therefore, all six mutant genes DP(98), DP(126), DP(159), DP(164), NDP(108) and NDP(149) are situated within this region.

The overall results of deficiency mapping (see Figure 4.5) are consistent with the physical mapping results.

4.2.4 *IN SITU* HYBRIDIZATION

Materials and Methods

Since the mutants were derived from crosses in which *P* elements were mobilized, *in situ* hybridization was used to correlate the presence/absence of *P* elements in the region to which the mutations map with the mutant phenotypes. Lines DP(146), DP(98), DP(126), NDC(19) and DP(146) were assessed once for sites of insertion of *P* elements within six generations of their discovery.

The nine mutant chromosomes were backcrossed to *C(1)DX* SAM for more than 10 generations, then derived into homozygous strains and maintained for about 30 generations before assessing them for sites of insertion of *P* elements. Line NDC(19) is sterile when homozygous in females, so was maintained by backcrossing to *C(1)DX* SAM for more than

20 generations with occasional relaxation.

In addition, lines DP(164)W and DP(146)W, established from wildtype revertant flies from mutant lines DP(164) and DP(146), and recombinant lines NDC(19) + *ct*^{6} *t*^{2} *v f car* and DP(146) + *ct*^{6} *t*^{2} *v f car* derived from the mapping experiment, were also assessed for sites of *P* element insertion.

The larvae were raised at 18°C in cornmeal-agar-molasses medium. The technique of *in situ* hybridization used was based on the method described by Shrimpton *et al.* (1986), and Leigh Brown and Moss(1987), and is given in detail in the Appendix.

Plasmid pπ25.1 (O'Hare and Rubin, 1983) was used as the *P* element probe, labelled with biotinylated dATP (bio-7-dATP, BRL) by nick translation. Hybridization was detected using the ABC kit (BRL) and visualized with horseradish peroxidase (diaminobenzidine).

Cytological positions (sites) of *P* element homology were recorded only for the *X* chromosome of each line. Each slide was examined twice at magnification 630 under oil immersion, and elements assigned to bands on photocopies of Lefevre's (1976) photographic map of the salivary chromosomes . Five to ten slides were prepared and scored from each line (mutant or wildtype). Common sites, present in all individuals within a line, and total sites, which include all sites present at least once in the sample, were summarized for each line on a Bridges' (1938) polytene map. The common sites were ascertained by rereading slides.

Results

The results of the complementation tests have shown that these mutant genes are located at two or three loci. NDC(19) is closely linked to *y*, and DP(146) is approximately 0.17 cM from it, whereas DP(98), DP(126), DP(159), DP(164), NDP(108) and NDP(149) are at the same locus, about 4.7 cM from the *ct* locus. Therefore, only the first 9 sections of the *X* chromosome were diagnosed for insertion sites of *P* elements. The common sites (sharp solid triangle) and total sites (arrow) for each mutant in the 9 section regions of the *X* chromosome are presented in Figure 4.6.

1)NDC(19): Figure 4.6a shows insertion sites of *P* elements on the tip of the *X* chromosome of recombinant + ct^6 t^2 *v f car* NDC(19). One of the two common sites located at region 1A-1B6 is most likely associated with mutant gene NDC(19). This insertion was detected in the early *in situ* hybridization analysis (see Figure 4.7). The *X* chromosome of NDC(19) originated from the inbred SAM stock, which harbours no defective or autonomous *P* elements, so this site is a *de novo* insertion. This mutant is stable, and so may be caused by an insertion of a defective *P* element.

2)DP(146): Figure 4.6b shows the common and total sites of *P* element insertions for mutant DP(146), combining results from the early (see Figure 4.8) and recent analysis. Figure 4.6c gives the pooled sites of insertion from three wildtype lines derived from mutant DP(146) in

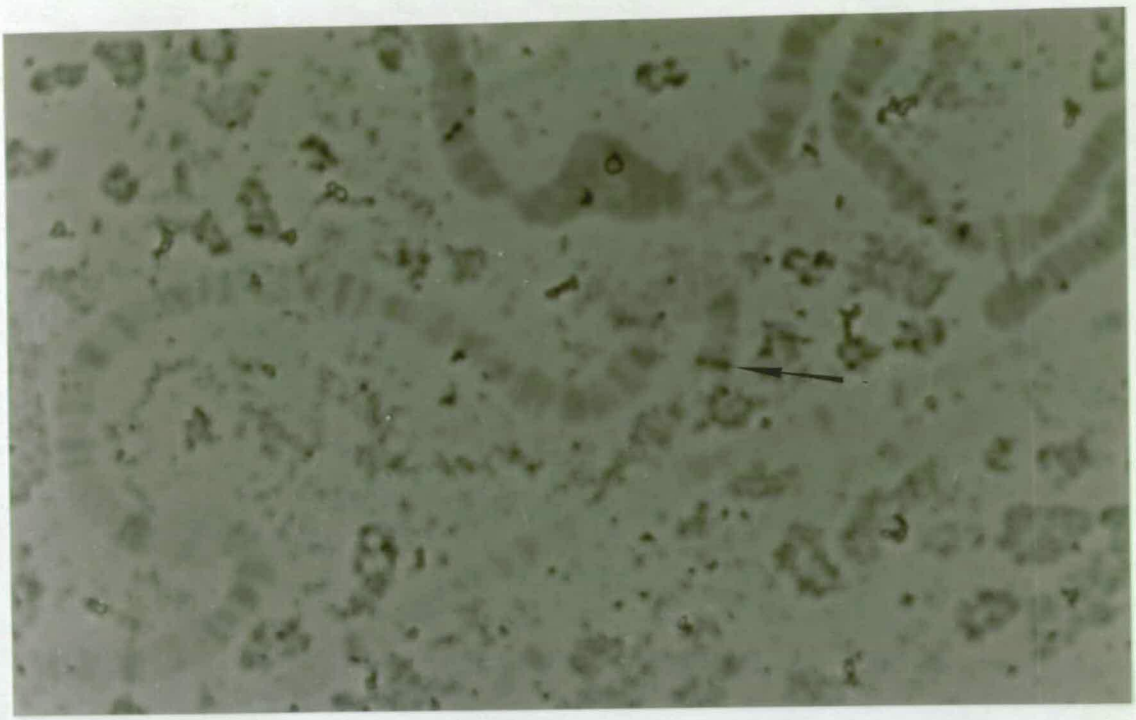


Figure 4.7 The *P* element insertion in the region 1A-1B of the NDC(19) *X* chromosome may be associated with this mutation. *In situ* hybridization analysis was performed at generation 4 after this mutant was found.

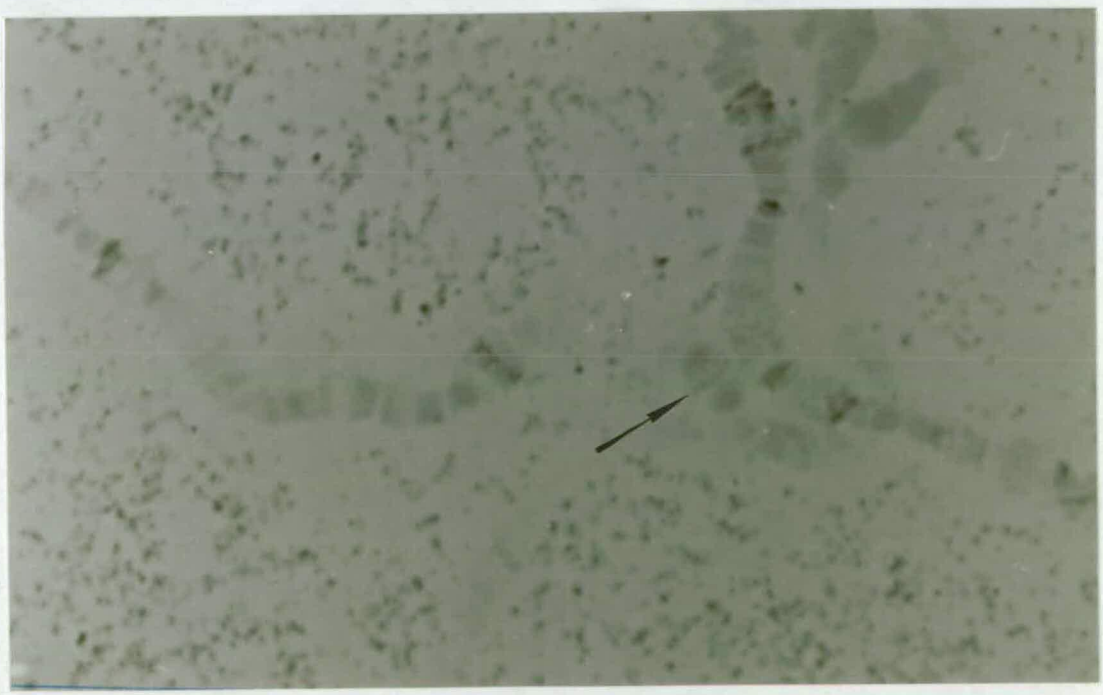


Figure 4.8 The *P* element insertion in the region 1B of the DP(146) *X* chromosome is associated with the mutation. This *in situ* hybridization was performed at generation 6 after the mutant line was discovered. Subsequent *in situ* hybridization at later generations confirmed this was a common insertion site for this mutant line.

different generations. Mutant DP(146) shares two common sites with its wildtype revertants, at bands 2E and 7B. However, the common site of insertion at band 1B is unique to the DP(146) mutant phenotype, and is not present in the wildtype revertants. This has been repeatedly confirmed by the observation that most (nine out of ten) of revertants (wildtype) of recombinant DP(146) + ct^6 t^2 *v f car* has no *P* element insertion at this site. This strongly suggests that the DP(146) mutation was induced by the insertion of a *P* element at band 1B. The instability of this mutant during maintenance supports this deduction. The map distance of DP(146) is 0.17 cM, but its physical position is likely to be close to the *y* locus. Deficiency mapping has crudely shown that DP(146) is located in between 1B04 or 06 and 1B10. Compared with insertion sites of other mutants, it appears that this site is a *de novo* insertion.

3) DP(98), DP(126), DP(159), DP(164), NDP(108) and NDP(149): Deficiency mapping indicates that these mutant genes are situated within the chromosomal bands of 8A04-8C06. However, none of these mutants have a common site of insertion in this region, but occasionally (uncommon sites) there is an insertion found within this region for mutants DP(98) (8C), DP(159) (8B1), DP(164)W (8C), NDP(108) (8C) and NDP(149) (8C). The wildtype strain NDP(106) has a common site of insertion at position of 8B. Although some wildtype flies were screened from mutant DP(164), there is no site of insertion of *P* elements that relates to this mutant gene within the corresponding region (see Figure 4.6j). Overall these data provide no direct indication that these mutations are associated with insertion of *P* elements. Given that these six mutations occurred at the same locus and were derived from a *P* strain

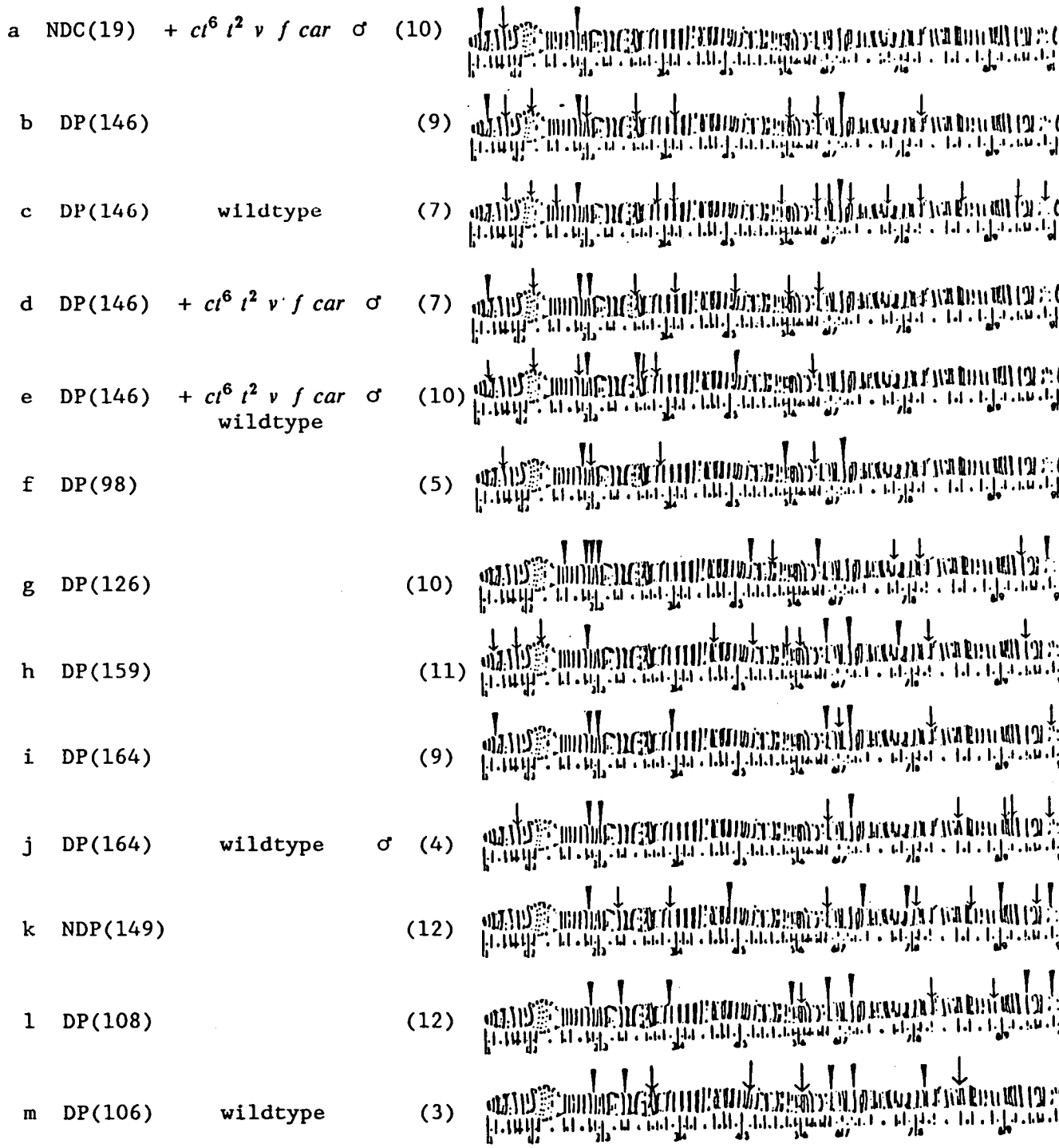


Figure 4.6 Insertion sites of *P* elements on the first 9 sections of the *X* chromosome. Sharp solid triangles (▼) and arrows (↓) depict the common sites and total sites over all salivary chromosomes assessed, respectively. ♂ means salivary chromosomes are from male larvae only, otherwise from male and female larvae. The number in the bracket is the sample size.

X chromosomes which previously harboured about 12 copies of *P* elements, these mutants are most likely caused by precise or imprecise excisions of *P* elements. Imprecise excisions leaving a defective *P* element less than 500 bp would not be recognizable by the method of *in situ* hybridization. The results of *in situ* hybridization in the early generation (G4) of lines DP(98), DP(126), DP(164) showed no indication of insertion of *P* elements within this region. The sites occasionally observed in mutants DP(98), DP(159), DP(164)W, NDP(108) and NDP(149) might have been inserted after the generation of the mutant lines. This is supported by the observation that all these mutants were generally stable during maintenance.

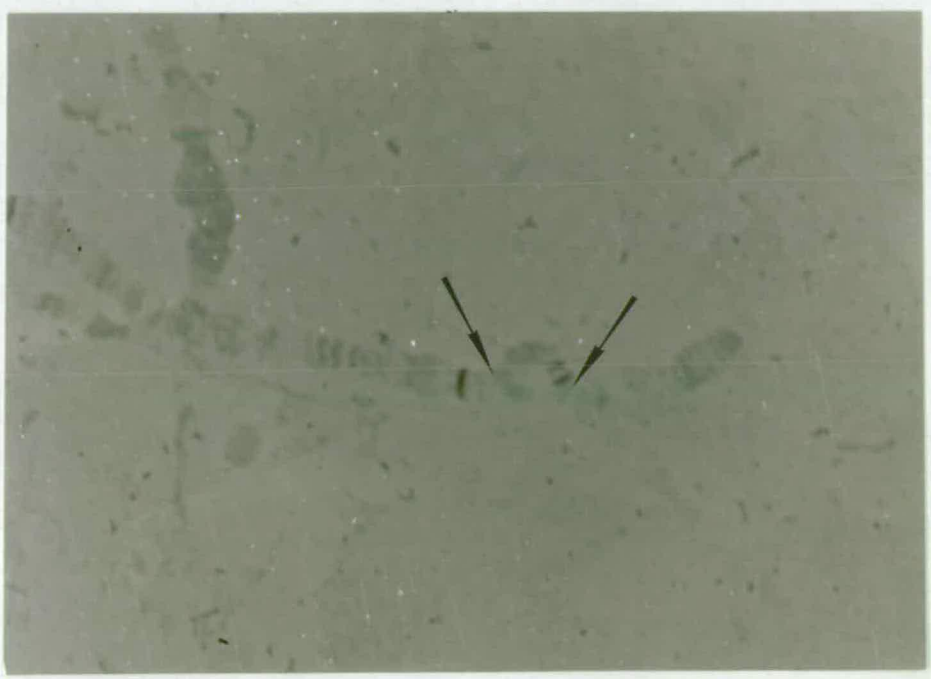


Figure 4.9 Translocation of the region of 12C-13B into 3A within mutant DP(98) *X* chromosome against SAM *X* chromosome in a heterozygous female larva.

4.3 DISCUSSION

Allelism: Although the physical map position of the mutant gene DP(98) is about 1 cM away from the other five mutant genes of Group A mutants, the allelism test has shown that it is an allele of the same locus. The reason for this biased estimate may be due to the instabilities of *P* elements it harbours on the *X* chromosome, which decrease viability of this mutant. The original mutant has been altered by a subsequent translocation in which region of 12C-13B has been inserted into 3A (see Fig. 4.9). The breakpoints (12C and 13B) happen to be previous sites of *P* elements, suggesting this translocation was caused by instability of *P* elements harboured by this mutant.

The allelism test showed the six mutant genes of Group A mutants are at the same locus, but the mean sternopleural bristle scores of heterozygous females of these mutants against wildtype are significantly diverse (see Chapter 3), while homozygous females of these six mutants display similar effects on the bristle scores. This may suggest they are different alleles of the same locus.

The map position of DP(146) is very close to NDC(19). Because DP(146) is very unstable, any reversion to the wildtype which occurred during mapping would have biased the estimation of its map distance. The disproportionate number (n_1) of mutants among recombinants of $y+$ compared to that (n_2) of wildtypes among recombinants of $+ ct^6$, suggests

the estimate of 0.17 cM for DP(146) to the *y* locus could be an overestimate. A conservative value may be calculated to be 0.03 cM, based on the number of mutants among recombinants of *y* + , e.g., $n_1+n_2=2$, 2/584×20=0.03. The deficiency mapping has more precisely indicated that mutant genes NDC(19) and DP(146) are within regions of 1B2;1B4-6 and 1B4-6;1B10 respectively. NDC(19) is phenotypically like *scute*, and may be a mutation in the *achaete-scute* region 1B1-4. However, the phenotype of DP(146) does not resemble *scute* mutations. Therefore it appears NDC(19) and DP(146) are closely linked but are at separate loci with some epistatic effects.

P element-induced mutations: The *X* chromosomes of all mutants except NDC(19) discovered in the early experiment (see Chapter 2) were derived from the highly inbred Harwich stock, which possesses roughly 40 copies of *P* elements. The original Harwich stock (outbred) has a common insertion site at cytological position 8C on the *X* chromosome. This site was occasionally present in four of the six mutants (DP(98), DP(159), NDP(108) and NDP(149)) with similar sternopleural bristle scores for which the mutant phenotype mapped to a single locus. This site was also observed in wildtype revertants of DP(164), wildtype revertants of DP(146) and mutant DP(146). It is commonly observed that mutations arising on *P*-derived chromosomes under conditions causing *P* element movement result more frequently from excision than insertion of *P* elements. Although the *in situ* hybridization analysis provides no direct evidence that the six mutants with a similar phenotypic effect on sternopleural bristle scores are caused by excisions of *P* elements, the similarity in the map position and phenotypic effects of these mutant genes, and common origin of the parent *X* chromosome, suggests

they most likely result from excision of *P* elements at salivary band 8C, which may be a hot-spot for excisions and insertions of *P* elements. The conjecture that these mutant genes are different alleles at the same locus (see section 4.3) is consistent with the hypothesis that they are caused by independent excisions of *P* elements at the same site. The combined physical and deficiency mapping and *in situ* hybridization results suggests that mutations NDC(19) and DP(146) were caused by separate *P* element insertions within the region 1A;1B10.

Quantitative genetic mutations of a single locus: The method used to generate and detect loci underlying a quantitative trait is unlike those used by other workers to map quantitative trait loci. These mutations were caused by *P* element mutagenesis induced by dysgenic or nondysgenic crosses. The methodology used to map these mutant genes is similar to that of Thoday (1961) and Shrimpton and Robertson (1988a,b), but in this case mutations are at single loci, not the larger "factors" or "chromosomal segments" identified by the techniques of resolving quantitative traits into Mendelian factors and mapping them by using either physical markers or RFLPs. Quantitative genetic mutations caused by *P* element insertions may be further investigated at the molecular level by cloning the loci into which the *P* elements have inserted. Mutant genes DP(146) and NDC(19) have pleiotropic effects on two bristle traits and viability. If NDC(19) is an allele of *achaete-scute*, it is straightforward to determine the nature of this mutation at the molecular level by cloning and restriction mapping, as *achaete-scute* has been cloned (Campuzano *et al.*, 1985). Since DP(146) also appears to be caused by an insertion of *P* element, it is possible to clone this bristle locus.

4.4 SUMMARY

Eight polygenic mutations affecting bristle scores induced by *P* element mutagenesis following dysgenic and nondysgenic crosses were mapped on the *X* chromosome of *Drosophila melanogaster*. Six mutants with similar low sternopleural bristle scores of about 15 are allelic with a map distance of approximately 24.7 cM, 4.7 cM from the *ct* locus. Deficiency mapping shows these mutant genes are within chromosomal bands 8A4;8C6. The other mutants NDC(19) and DP(146) have extremely low abdominal and sternopleural bristle scores of 11.5 and 14.9, and of 10.3 and 12.5, respectively, and both genes are closely linked to the *y* locus. The test of allelism and deficiency mapping indicate they are closely linked but at separate loci within chromosomal bands 1B2;1B4-6 and 1B4-6;1B10, respectively, with some epistatic effects. Mutant NDC(19) is probably a mutation of the *scute* locus. *In situ* hybridization analysis suggests these two mutants are associated with insertion of *P* elements within the chromosomal region 1A;1B10, but the other six mutants probably resulted from excision of *P* elements at the site of 8C.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSION

5.1 TRANSPOSABLE ELEMENT-INDUCED QUANTITATIVE VARIATION

Estimates of *P* element-induced mutational heritabilities of abdominal and sternopleural bristle scores on the *X* chromosome in this experiment are exceedingly high. A single generation of exposure to *P* element mutagenesis is about one hundred times more powerful than 1000r X-ray (which is $3\times10^{-3}V_e$/1000r/generation, Mackay, 1987) in inducing variation for abdominal bristle traits. *P* strain *X* chromosomes are eight times more mutable than *M* strain *X* chromosomes, and dysgenic crosses are about three times more effective than nondysgenic crosses in generating quantitative variation. This result is similar to those of Mackay (1986, 1987), who demonstrated similar levels of *P* element-induced mutational variation on the second chromosome, and emphasized the power of *P* element mutagenesis in generating quantitative variation.

5.2 EFFECTS OF MUTATIONS AFFECTING QUANTITATIVE TRAITS

The effects of the ten mutant *X* chromosome lines on bristle traits (see Chapter 3) are diverse, ranging from 1.2 to 5.5 σ_p in

heterozygous females, and 2.3 to 9.1 σ_p in hemizygous males. This result indicates that the effects mutations affecting quantitative traits among different loci are highly variable, and possibly leptokurtic. Robertson (1967) first pointed out that since there are a very large number of loci at which mutation can occur, the most likely shape of the distribution of gene effects is leptokurtic with high variance. Alleles with large effect on the selected trait have been often observed in selection lines. In selection experiments for bristle scores of Drosophila, *bobbed* (Clayton and Robertson, 1957 (presumedly); Frankham *et al.*, 1978), *scabrous* (Jones *et al.*, 1968; Yoo, 1980b), *scute* (Yoo, 1980b), and probably *Delta* (Yoo, 1980b) were detected; and the *dwarf* (Robert and Smith, 1982), *obese* and *pygmy* (Falconer, 1989a) mutants have been found in selected populations of mice. It is likely these alleles arose during selection by mutation (Hill, 1982a,b). However, it has been shown (Frankham and Nurthen, 1981; Yoo, 1980a) that rare genes (*i.e.smooth* in a natural Australian Drosophila population) with large effects are also present in natural populations.

The substantial increase of variance for two bristle traits in the *P* strain *X* chromosome populations were apparently caused by the eleven mutant lines with large effects, indicating a very high variance of mutant effects. It has been well documented (Clayton and Robertson, 1957; Thoday and Boam, 1961; Roberts, 1966; Falconer, 1971; Sheldon and Milton, 1972; Enfield, 1977, 1980; Yoo, 1980a; Sheldon and Evans, 1981) that in selected populations a rare gene of large effect cause a perceptible jump in response, followed by a period of small increment in response, and accompanied by large variation among replicates in

response. Similar observations have also been made in selected lines following dysgenic (M ♀♀ × P ♂♂) (Mackay, 1985; Pignatelli and Mackay, 1989) and nondysgenic (P ♀♀ × M ♂♂) (Torkamanzehi *et al.*, 1988; Pignatelli and Mackay, 1989) crosses.

Although mutant alleles of large effects at quantitative trait loci contribute dramatic variance to the population which can be utilized by selection, their effects on equilibrium genetic variation depends on whether stabilizing selection operates on the trait they affect, and on whether they have associated fitness effects. The theory of Keightley and Hill (1988) predicts that with stabilizing natural selection, the variance at equilibrium contributed by mutations with large effects are less than that contributed by mutations with small effects since the former are eliminated rapidly by selection.

5.3 PLEIOTROPY

Two mutants characterized in this project have pleiotropic effects on abdominal and sternopleural bristle scores and viability. The direction of effects of each mutant is similar, that is to decrease both bristle number and viability of flies. The effects on each character are variable, and summarized in Table 5.1. The reduction in viability, 1-v, where v is the relative viability (see Chapter 4) of each mutant, was derived by assuming the viability of wildtype is 1.

Correlations between the effects of mutations on quantitative traits and fitness have long been of interest (reviewed by Mackay, 1989). Clayton and Robertson (1957) extracted and made homozygous

individual chromosomes from Drosophila lines selected for high and low abdominal bristle scores. Many second and third chromosomes were homozygous lethal, with heterozygous effects on the selected trait in the direction of selection. One of the homozygous lethal lines had a heterozygous effect of 22 abdominal bristles. This observation was subsequently repeated in several other long-term Drosophila selection lines (Latter and Robertson, 1962; Frankham *et al.*, 1968; Hollingdale, 1971; Madalena and Robertson, 1975; Frankham, 1980a; Yoo, 1980b). It has been argued these chromosomes extracted from selected lines may contain mutations at single loci with pleiotropic effects on bristles and fitness, since no instance of recombination between the bristle effects and the lethal has been reported. In addition, major morphological mutations which are found in selection lines often have pleiotropic effects on the selected quantitative trait and fitness. The *bobbed* mutation arose in Drosophila lines selected for decreased abdominal bristle score (Clayton and Robertson, 1957; Frankham *et al.*, 1978; Frankham, 1980a), and *bobbed* females are less viable and have longer development times than wildtype (Ritossa, 1976). The *dwarf, obese, pygmy* and high growth gene in mice have occurred in selection lines, and are either sterile or have reduced fertilities when homozygous (Falconer, 1981; Bradford and Famula, 1984). The *halothane* gene in pigs and double muscling gene in cattle reduce viability and fertility (Hanset, 1982; Webb *et al.*, 1982). Evidence such as this leads to the conclusion (Mackay, 1989) that the more extreme the effects of mutation on the trait, the more severe the reduction in fitness. Relative viabilities of DP(146) and NDC(19) were measured indirectly (see Chapter 4), they are 0.712 and 0.140 (assuming the wildtype is 1),

respectively. Mutant DP(146)NAB is lethal in homozygous females. The order of effects of these mutants on bristles is: DP(146) < NDC(19) < DP(146)NAB, in agreement with this hypothesis.

The correlation between abdominal and sternopleural bristle scores of *Drosophila melanogaster* has been estimated from various populations, e.g. Sheridan *et al.*, (1968) estimated the phenotypic and genetic additive correlation between the traits to be (r_p=) 0.14, and (r_a=) 0.41, respectively. Correlated responses for these two characters were found in all four single trait directional selection lines of Davies and Workman (1971), and in all cases were in the same direction as the direct response to selection in the other character. Davies (1971) mapped these abdominal and sternopleural effects separately, and concluded the correlated responses were from closely linked genes, rather than from genes with pleiotropic effects on both characters.

TABLE 5.1 Pleiotropic Effects of Two Extreme Mutants

Mutant Line		Abdominal Bristle Effect (σ_p)	Sternopleural Bristle Effect (σ_p)	Viability
DP(146)	♀	-2.53	-1.20	-0.2884
	♂	-3.58	-2.57	
NDC(19)	♀	-3.63	-3.14	-0.8601
	♂	-5.16	-7.23	

There are some defects with his analysis (McMillan and Robertson, 1974), shown by several identified regions with negative effects on otherwise positive chromosomes, which must cast some doubt on the

conclusions.

The two mutant lines described in Table 5.1 occurred after a single generation of mutagenesis, and the mutations map to different chromosomal positions: NDC(19) is within the 1B2;1B4-6 region, and DP(146) is within the 1B4-6;1B10 region of the *X* chromosome. No recombinant between the abdominal and sternopleural bristle effect has been observed for these mutants; they are almost certainly point-mutations with pleiotropic effects.

It has been widely realized that the same genetic correlation can arise, either because a large number of loci each has relatively small pleiotropic effects, or a few pleiotropic genes or tightly linked loci each has a large effect. The work of Bohren *et al.* (1966) have shown that asymmetry in response to selection may result from the relative change in frequency of genes contributing positively and negatively to the covariance of the traits. For genes with large effects, Bohren *et al.* (1966) note this will lead to asymmetric response in the very first generation of selection.

Turelli (1985) deduced that if the pleiotropic effects of mutants are under multivariant stabilizing selection, the univariant prediction of equilibrium genetic variance tends to be biased upward, because the force of selection on a single character can greatly be strengthened by selection on other phenotypically correlated characters (Lande and Arnold, 1983). Alternatively, if pleiotropic effects are involved in balancing selection rather than phenotypic stabilizing selection, pleiotropy can be a potent force maintaining genetic variability (Gillespie, 1984).

In addition, it is commonly observed that mutations with a major

morphological effect on one trait also have a multitude of pleiotropic effects on other traits, often including reduction of fitness (see above). This can lead to a selection plateau in which genetic variability remains if such alleles affect the selected trait (Clayton and Robertson, 1957). This is due to the maintenance in the selected population of homozygous lethal or sterile genes with heterozygous effect on the selected trait, so that artificial selection is balanced by natural selection.

5.4 INSERTIONAL MUTATIONS AND QUANTITATIVE TRAIT LOCI

Questions about the nature of the genes causing quantitative variation have long been of interest. In the classic theory it is assumed genes affecting quantitative traits are those having only small and equal effects. However the molecular bases of quantitative trait loci are unknown. It has been proposed that quantitative genetic variation could be a consequence of the secondary effects of genes with other major functions (discussed by Falconer, 1989a). This is supported by the fact that most mutations in Drosophila with major morphological effects on one trait also have pleiotropic effects on other traits (see Lindsley and Grell, 1968). The second possibility is that loci affecting quantitative traits have a spectrum of allelic effects, some so large as to cause the recognition of the locus as a major gene affecting the trait, others sufficiently small that segregating "isoalleles" give rise to quantitative variation (Robertson, 1985; Mackay, 1985). A third proposal is that quantitative trait loci are

modifiers of major gene expression. It has been demonstrated experimentally that isoalleles of major loci can have essentially wild-type effect (e.g. Muller, 1935; Green, 1959; Tsubota and Schedl,1986), and modifier loci affect the expression of major genes (e.g. Milkman, 1970; McDonald and Ayala, 1978; Laurie-Ahlberg *et al.*, 1982). A popular hypothesis proposed by Wilson (1976) and Mukai and Cockerham (1977) is that most quantitative variation is caused by allelic variation or mutations of the regulatory regions of structural genes, resulting in changes of timing and/or expression of a gene product, rather than a qualitative change in the protein. Since the rate of morphological divergence among mammals is much too great to be accounted for by the rate of divergence of structural loci, Wilson (1976) therefore argued that regulatory mutations which alter the expression of genes are instrumental in morphological evolution. Comparing the spontaneous mutation rates of viability polygenes and isoenzyme structural loci on the second chromosome of *Drosophila melanogaster*, Mukai and Cockerham (1977) suggested that regulatory mutations are responsible for variation in Drosophila viability. Regulatory changes could result from an alteration in the pattern of transcription by nucleotide substitution or small insertions / deletions in the control sequences outside the protein coding region of quantitative trait loci, insertions or by deletions of transposable elements.

The discovery of the nature of the *hairy* locus provides one piece evidence to support this "regulatory" hypothesis. The *hairy* locus was originally identified as a mutant allele with a major morphological effect on the number of microchaetae. This complex gene comprises two regulatory regions and one structural region (Ingham *et al.*, 1985).

Mutations of different regulatory regions result in the variety of phenotypes. In addition, Botas *et al.* (1982) have shown the *hairy* locus also regulates the unlinked *achaete-scute* complex. Mutations in the regulatory regions of the *achaete-scute* complex also cause quantitative variation in bristle phenotypes. Genetic analysis has subdivided the complex into five regions: *achaete*, *scute* α, lethal of *scute*, *scute* β (Muller, 1955; Garcia-Bellido, 1979) and *scute* γ(Dambly-Chaudiere and Ghysen, 1987; Ghysen and Dambly-Chaudiere, 1988). The whole *AS-C* complex has been cloned recently (Campuzano *et al.*, 1985) and its transcription pattern analyzed. At least eight different transcription units have been recognized in the complex (Campuzano *et al.*, 1985; Villares and Cabrera, 1987). These transcriptions (T1-T8) are separated by long stretches of apparently untranscribed DNA. Interestingly, most lesions associated with the nonlethal *ac* or *sc* phenotypes have been localized within these noncoding regions. The phenotypic effects of deficiencies in this region depend on the proximity of the breakpoint to the coding region, the closer the breakpoint, the more severe the phenotypic effect. The deficiency breakpoints as far away as 50 kb from the *sc* coding regions have a weak *sc* phenotype (Ruiz-Gomz and Modolell, 1987). Moreover, it appears that the *cut* locus has similar properties (Johnson and Judd, 1979).

Most of the moderately repeated components of the eukaryotic genome consists of transposable element (TE) sequences. Rubin (1983) inferred that most "spontaneous" mutations in Drosophila have been caused by TE insertions, and many insertional mutations have been described in maize and yeast. The well-documented genetic and molecular analysis of TE-induced mutation and their reversions in maize,

Drosophila and yeast have shown that insertion of TEs at or near a functioning gene may modulate that gene's expression.

Tsubota and Schedl (1986) observed that eighteen new mutations in the 5' control region of the *rudimentary (r)* gene generated by insertion and excision of *P* elements alter the amount of transcript produced. The developmental timing or tissue specificity of expression may be altered by TE insertion (Chia *et al.*, 1986). The position of the inserted TE within the gene and the structure of the inserted sequence may influence the severity of the mutational effects. Imprecise excisions of TEs usually generate wild-type pseudorevertant phenotypes (Rubin, 1983; Tsubota and Schedl, 1986); and insertion of a second TE at or near the first may enhance the severity of the mutant phenotype (e.g. Rubin, 1983; Engels, 1988; Mount *et al.*, 1988). An unlinked gene can suppress (restore to wild type) or enhance (create a more extreme phenotype) insertional mutations (Modolell et al., 1983; Rubin, 1983; Fassler and Winston, 1988; Mount *et al.*, 1988). Insertional mutations are less common in coding regions, and such insertions in coding regions usually cause extreme phenotypes, or nulls (Chia *et al.*, 1986).

There is no doubt that TEs induce quantitative variation (see above 5.1). Two of eight polygenic mutations induced by *P* element mutagenesis reported in this thesis are associated with insertion of *P* elements within the region 1A-1B10 of the *X* chromosome, while the rest probably result from excision of *P* elements. Selection for inebriation time (Frankham, Torkamanzehi, and Moran, unpublished results) carried out in microinjected *P*-element lines that had previously no *P* element, showed a significant response relative to

the *P* element-free base population.

Do TE insertions in natural populations affect quantitative variation? A sample of 36 *X* chromosome lines with isogenic autosomes (see Miyashita *et al.*, 1986, for details of their derivation) from two natural populations (Raleigh and Texas) were assessed (T.F.C. Mackay and C.H. Langley, unpublished data) for abdominal and sternopleural bristle scores and insertion sites within the *y-ac-sc* region. There was a highly significant reduction in scores of both bristle characters for haplotypes containing insertions compared to non-insertion haplotypes: 1.62 sternopleural and 1.18 abdominal bristles, respectively. This is the first evidence that insertions in natural populations can cause quantitative variation.

Mutants NDC(19) and DP(146) are associated with insertions of *P* elements, so by using the *P* element as a probe these mutant genes including the inserted *P* elements can be isolated and cloned from the DNA libraries established from these mutant lines. In addition, NDC(19) is phenotypically and cytogenetically like a mutation of the *scute* locus, and the *scute* region has been cloned (Campuzano *et al.*, 1985; Villares and Cabrera, 1987). This facilitates cloning and sequencing of this bristle locus. When the wildtype DNA sequences of mutants DP(146) and NDC(19) can be recovered, they will provide important information about QTLs at the molecular level. Nevertheless, in order to evaluate hypotheses concerning the nature of quantitative variation, more QTLs must be identified and cloned in *Drosophila melanogaster*. The availability of a new stable genomic source of *P* transposase (P[ry^+ Δ2-3], Robertson *et al.*, 1988) enables single insertional mutations to be generated and stabilized in a desired genetic background. This

transposase-producing element can be used to mobilize the P[lArB] plasmid (Bellen *et al.*, 1989), containing the *P-lacZ-hsp* 70 fusion gene, the Adh^{+} and ry^{+} genes, and the Bluescript plasmid vector (Bellen *et al.*, 1989). This technique facilitates identification of transformants in a ry^{-} background and subsequent cloning of genomic sequences adjacent to the site of insertion. Once loci capable of mutating to alleles affecting quantitative traits in Drosophila are identified by insertional mutagenesis, it will be possible to determine their contribution to genetic variation of these traits in natural populations and the nature of quantitative variation at the molecular level.

APPENDIX

Method for *In situ* Hybridization of *Drosophila Melanogaster* Salivary Chromosomes Using Biotinylated DNA

1. Procedure

A. Preparation of Salivary Chromosomes

1. Dissect salivary glands from larvae in 45% acetic acid, removing as much of the fat bodies, salivary gland membrane, and salivary ducts from the glands as possible. Dissect on a depression slide using needles and fine forceps.
2. Transfer the glands to a fresh drop of 45% acetic acid for a minute or two.
3. Transfer the glands into a drop of "1:2:3" solution on a siliconized coverslip. Keep the glands there for 4 or 5 minutes.
4. Pick up the coverslip containing the salivary glands with a slide treated in "SSC-Denhart" solution.
5. Spread the chromosome by gently making streaks over the coverslip with a needle, or by gentle pressure from the forefinger. Spread them further by hammering carefully with the rubber end of a pencil.
6. Examine the slides under phase contrast at 100 power. Focusing initially on the edge of the coverslip helps locate the

salivaries.

7. Refrigerate the slides overnight to flatten the salivary chromosomes.

8. Remove coverslips as follows:

   a. Using tongs, dip slides into liquid nitrogen for 15 seconds (or until slides stops "fizzling").

   b. Pop the coverslip off slide with a razor blade, using a quick motion.

   C. Immediately place slides in cold 95% ethanol(keep it at -20°C before use) at room temperature for at least 15 minutes. Slides may be stored for several weeks in 95% ethanol before *in situ* hybridization.

9. Remove the slides from the 95% ethanol and let them dry in the air. Slides may then be stored in the refrigerator for months before using.

B. Prehybridization treatment

1. Treat the slides in 2XSSC at 65°C for 30 minutes to remove basic proteins.

2. Dehydrate the slides by placing them sequentially in two baths of 70% ethanol 1 for 5 minutes, at room temperature, and then in 95% ethanol for 5 minutes, again at room temperature.

3. Let the slides dry in the air or fumehood.

4. Denature the slides in 0.07N NaOH for 2 minutes, at room temperature.

5. Rinse the slides sequentially in 3 baths of 2XSSC for 5 minutes each at room temperature.

6. Dehydrate again in 2 baths of 70% ethanol for 5 minutes each, followed by a bath in 95% ethanol for 5 minutes, all at room temperature.

7. Remove the slides from the 95% ethanol and let them dry in the air or a fumehood.

8. Mark the position of coverslip on other side of each slide using color pen in waterproof ink.

C. Hybridization

1. Apply 15 to 20 μl of the hybridization probe to a siliconized coverslip, and pick up the coverslip with the slide at the position where the salivary is located.

2. Hybridize overnight at 37°C in a moist chamber in order that the preparation not dry out under the coverslip.

D. Washes and detection

1. Wash slides sequentially in 2 baths of 2XSSC for 10 minutes each, at 37°C. Meanwhile move the slide frame gently to wash off the coverslip.

2. Wash slides sequentially in 2 baths of 2XSSC for 10 minutes each, at room temperature.

3. Wash slides for 5 minutes in PBS at room temperature.

4. Mix "ABC" complex by adding 10 μl of each of the two components in the ABC kit to 1.25ml of 50mM Tris, pH 7.6, containing 4% bovine serum albumin (BSA). Let the mixture stand for 5 minutes before use.

5. Shake off PBS from slides but do not let them dry out. Add 20 μl of "ABC" mixture to a siliconized coverslip, pick it up with the slide like step C1, incubate the slides at 37°C in a moist chamber for 30 minutes.

6. Wash the slides in three sequential baths of PBS for 10 minutes each. The coverslips should float off; nudge them loose if necessary.

7. Make up a fresh solution of DAB (3,3' diamobenzidine tetrachloride), at a concentration of 0.5 mg/ml in 0.05 Tris, pH 7.6. Immediately before use, add 10 μl of 30% hydrogen peroxide for each 5 ml of the solution.

8. Apply 20 μl of DAB solution to a siliconized coverslip, and pick it up with the slide like C1. Incubate the slides in moist chamber for at least three hours or overnight at 37°C.

9. Wash the slides for 5 to 30 minutes in PBS.

10. Stain the slides for 1 minutes in a 8% solution of Giemsa in 0.05M Tris buffer, pH 7.6. This stain solution should be freshly prepared.

11. Rinse slides in distilled water and dry at room temperature.

12. Rinse the slides with xylenes before mounting them with permount and coverslips. These slides can be stored for years at room temperature.

E. Nick translation using Biotinylated-dATP.

1. Make up 50 μl of following components (BRL nick translation system products):

		Final concentration
1) solution A1	5μl	
containing: 0.2 mM dCTP,		20μM
0.2 mM dGTP,		20μM
0.2 mM dTTP,		20μM
500 mM Tris-HCl (pH 7.8),		
50 mM $MgCl_2$,		
100 mM 2-mercaptoethanol		
100 μg/ml nuclease-free BSA		
2) Biotinylated-dATP		
0.4 mM	7.5μl	60μM
3) *P*π25.1 DNA		
0.5mg/ml	2μl	20μg/ml
4) solution C	5μl	
containing: 0.4 u/μl DNA polymerase I,		0.04u/μl
40pg/μl DNAse I,		
50mM Tris-HCl (pH 7.5),		
5 mM Mg-acetate,		
1 mM 2-mercaptoethanol,		
0.1mM PMSF,		
50% (v/v) glycerol,		
100 μg/ml nuclease-free BSA		
5) distilled H_2O	30.5μl	
	50μl	

2. Mix thouroughly but gently and centrifuge briefly in a microfuge to bring liquid to the bottom, then incubate at 15°C for 90

minutes.

3. Add 5 μl 0.5M EDTA (stop solution).

4. Boil it on a water bath for 10 minutes.

5. Add 5 μl 3M NaAcetate and 100 μl cold 100% ethanol (keep it at -20°C before use), then put it in a freezer at -20°C for at least 1 hour.

6. Spin in a microfuge for 10 minutes and discard the supernatant, then wash the pellet with 300 μl 70% ethanol.

7. Let it the pellet dry, then vacuum dry at room temperature.

8. Resuspend at a DNA concentration of 20 μg/ml in following buffer:

1) distilled H_2O	60μl
2) 20XSSC	50μl
3) salmon sperm DNA	40μl
4) 50% dextran sulphate	100μl
5) Formamide	250μl
Total	500μl

9. This hybridization probe is stored at -20°C, and boiled before use.

2. Preparation of Solutions

Solutions are listed by the section where they are used.

A3. 1:2:3 solution:

1 part lactic acid: 2 parts water: 3 parts acetic acid

A3. Siliconized coverslip:

Drop coverslips into a 5% dimethyldichorsilane in chlotoform, then rinse 3-4 times in distilled water and keep in 100% ethanol.

A4. Treating slides in "SSC-Denhart" solution:

1. Incubate slides for 2.5 hours at 65°C in SSC-Denhart solution.
2. Dip the slides in distilled water for a few seconds.
3. Fix them in ethanol-acetic acid fixative (3:1) for 20 minutes at room temperature.
4. Air dry the slides and store them in refrigerator until use.
5. To make 2400 ml of SSC-Denhart solution, enough for 6-7 staining dishes of 30 slides capacity:

   360 ml of 20XSSC

   48 ml 1% (W/V) PVP 360 (polyvinylpyrrolidone)

   48 ml 1% Ficoll

   48 ml 1% nuclease-free Bovine Serum Albumin

   1896 ml distilled H_2O

6. 20XSSC (sodium chloride/sodium citrate):

   3M NaCl 175.2 gm/liter

   0.3M Na Citrate.$2H_2O$ 88.23 gm/liter

B4. 0.07N NaOH:

Add 1.4 g dry NaOH to 500 ml H_2O.

D4. 10XPBS(Phosphate buffered saline), pH 7.4:

1.3 M NaCl 75.92gm/liter

0.07M $Na_2HPO_4.7H_2O$ 18.76gm/liter

0.03M $NaHPO_4.H_2O$ 4.14gm/liter

D6. 50mM Tris, pH 7.6, containing 4% BSA:

Add 606 gm Tris to 100 ml H_2O.

Adjust pH to 7.6 with concentrated HCl.

Add 4gm BSA.

Aliquot 1.25 ml into Eppendorf tubes and freeze.

Thaw before use.

D7. DAB solution:

Add 5mg DAB(3,3' diamobenzidine tetrachloride) to 9.5 ml H_2O.

Add 500 μl 1M Tris, pH7.6.

Add 20 μl 30% hydrogen peroxide, just before use.

D8. 8% Giemsa solution in 0.05 M Tris buffer, pH 7.6:

12.5 ml 8% Giemsa solution

10 ml 1M Tris buffer, pH 7.6

177.5ml distilled H_2O

8% Giemsa solution:

Add 0.5 gm Giemsa to 33 ml glycerol.

Heat and stir it at 60°C for 2 hours in a fumehood.

Add 33 ml methanol after it is cool.

Mix well and filter it.

E3. 0.5M EDTA:

Add 186.1 gm disodium ethylene diamine tetraacetate•$2H_2O$ to 800 ml of distilled H_2O.

Stir vigorously on a magnetic stirrer.

Ajust the pH to 8.0 with NaOH (20 gm NaOH pellets).

Dispense into aliquots.

E8. 50% dextran sulphate:

Add 50 gm dextran sulphate into 50ml H_2O.

E8. Salmon sperm DNA:

Dissolve the DNA in water at a concentration of 10mg/ml.

Stir the solution on a magnetic stirrer for 2-4 hours at room temperature.

Shear the DNA by passing it several times through an 18-gauge hypodermic needle.

Boil it for 10 minutes and store at -20°C in small aliquots.

Boil it for 5 minutes before use.

Hybrid Dysgenesis-Induced Quantitative Variation on the *X* Chromosome of *Drosophila melanogaster*

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ABSTRACT

To determine the ability of the P-M hybrid dysgenesis system of *Drosophila melanogaster* to generate mutations affecting quantitative traits, *X* chromosome lines were constructed in which replicates of isogenic M and P strain *X* chromosomes were exposed to a dysgenic cross, a nondysgenic cross, or a control cross, and recovered in common autosomal backgrounds. Mutational heritabilities of abdominal and sternopleural bristle score were in general exceptionally high–of the same magnitude as heritabilities of these traits in natural populations. P strain chromosomes were eight times more mutable than M strain chromosomes, and dysgenic crosses three times more effective than nondysgenic crosses in inducing polygenic variation. However, mutational heritabilities of the bristle traits were appreciable for P strain chromosomes passed through one nondysgenic cross, and for M strain chromosomes backcrossed for seven generations to inbred P strain females, a result consistent with previous observations on mutations affecting quantitative traits arising from nondysgenic crosses. The new variation resulting from one generation of mutagenesis was caused by a few lines with large effects on bristle score, and all mutations reduced bristle number.

TO what extent do transposable elements (TEs) cause variation for quantitative traits? This question is important for understanding the basis of quantitative variation in natural populations, and also because TE-induced mutations at quantitative trait loci provide an experimental approach to mapping these loci and further characterizing them at the molecular level (BINGHAM, LEVIS and RUBIN 1981). Certainly TEs are common components of many eukaryotic genomes–in *Drosophila melanogaster* the five major categories of TEs make up at least 10% of the genome (RUBIN 1983; FINNEGAN and FAWCETT 1986)–and they are known to have a wide range of mutagenic properties which include creation of chromosome rearrangements, induction of null and lethal mutations, as well as more subtle regulatory changes (summarized by MACKAY 1989). GREEN (1988) has asserted that most spontaneous mutations in *D. melanogaster* are caused by insertions of TEs. Furthermore, the TEs of *D. melanogaster* have structural homology to retroviruses and other TEs in a variety of eukaryotes (FINNEGAN 1985), and evidence is accumulating that these elements are a major source of mutational variation in species other than *Drosophila* (*e.g.* LAMBERT, MCDONALD and WEINSTEIN 1988).

To determine the extent to which TE-induced mutations can cause variation for quantitative traits, it is necessary to compare the amount of genetic variation for the traits of interest in strains in which TEs have and have not moved, but which otherwise have a common genetic background. The difference in amount of genetic variance between these strains estimates the TE-induced mutational variance (V_m), from which the mutational heritability ($h_m^2 = V_m/V_m + V_e$), where V_e is the environmental variance) can be derived. Such experiments are feasible in *D. melanogaster* because the *P* and *I* element families transpose very frequently under conditions of hybrid dysgenesis (KIDWELL, KIDWELL and SVED 1977; BINGHAM, KIDWELL and RUBIN 1982; BUCHETON *et al.* 1984). Hybrid dysgenesis and associated germ-line *P* element transposition are induced in hybrid progeny of an interstrain cross of strain females without *P* elements to P strain males with multiple *P* element copies, but at much lower frequencies in the reciprocal nondysgenic cross of P strain females to M strain males. Hybrid dysgenesis-induced *P* element mutagenesis has in particular been used successfully to clone many genes (reviewed by KIDWELL 1986). However, application of P-M hybrid dysgenesis to the generation of polygenic mutations has proven rather less straightforward than generating mutations of large qualitative effect. P-induced mutants of large effect are readily observed in the F_2 progeny of dysgenic hybrids, but polygenic mutations of smaller effect are likely to be swamped by the variance for the trait segregating in the F_2 as a result of the interstrain cross.

Two experimental designs have been used to esti-

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mate the magnitude of hybrid dysgenesis-induced quantitative variation: response to artificial selection of inter-P and -M strain hybrids (MACKAY 1984, 1985; TORKAMANZEHI, MORAN and NICHOLAS 1988; PIGNATELLI and MACKAY 1989), and variation among P (YUKUHIRO, HARADA and MUKAI 1985; FITZPATRICK and SVED 1986) or M (MACKAY 1986, 1987; EANES *et al.* 1988) strain chromosomes contaminated with *P* elements by inter-P and -M strain crosses. Artificial selection lines often show patterns of response expected from the presence of mutations affecting the selected trait, with unexpected asymmetrical responses to divergent selection, greater variation in response among replicates than expected from drift, and increased heritability and phenotypic variance in those replicates exhibiting accelerated response (PIGNATELLI and MACKAY 1989). MACKAY (1985) suggested that P-induced mutational variance could be estimated from the average difference in response of selection lines established from dysgenic and nondysgenic crosses, on the basis of observations of accelerated responses of dysgenic selection lines, but not nondysgenic selection lines. However, these observations were not repeated by TORKAMANZEHI, MORAN and NICHOLAS (1988) or PIGNATELLI and MACKAY (1989), when it became clear that "nondysgenic" selection lines could also exhibit responses typical of the presence of new polygenic mutations. The repression of element transposition in nondysgenic F_1 hybrids apparently breaks down when they are mated *inter se* for several generations, so the response to selection of nondysgenic hybrids does not provide a transposition-negative control in these experiments. A. E. SHRIMPTON, T. F. C. MACKAY and A. J. LEIGH BROWN (unpublished data) determined the cytological locations of *P* elements in the selection lines of MACKAY (1985) and in the P strain used in their production, and showed that all dysgenic and nondysgenic lines had large numbers of new insertions.

The estimates of P-induced variation from difference in response to selection of dysgenic and nondysgenic hybrids are seriously biased downward, not only because transposition occurs in nondysgenic selection lines, but also because new mutations may be lost by drift or natural selection due to deleterious fitness effects. Accumulation of new P-induced mutations on chromosomes kept heterozygous against a balancer chromosome gives better estimates of total polygenic mutation rate because mutations are not lost. In general, chromosome lines which accumulate hybrid dysgenesis-induced mutations have decreased viability (YUKUHIRO, HARADA and MUKAI 1985; MACKAY 1986; EANES *et al.* 1989) and total fitness (FITZPATRICK and SVED 1986; MACKAY 1986), and exceptionally high mutational heritabilities for bristle traits of the order of 0.2 (MACKAY 1987). However, these experiments all differ according to whether P or M strain chromosomes were subjected to hybrid dysgenesis, and whether the background genotype was homogenized in a P strain background, an M strain background, or not at all. The various methods may lead to the production of different amounts of mutational variation. P strain chromosomes may be more susceptible to P-induced mutations than M strain chromosomes because excisions and rearrangements as well as transpositions can occur on the former, but only transposition will cause mutations in the latter. The process of placing the mutagenized chromosomes in a common background by backcrossing to a P or M strain has the advantage of allowing the detection of mutations of smaller effect than can be detected if the background remains segregating, but further mutations may occur on the target chromosomes during backcrossing.

To determine the effects of different procedures for generating chromosome lines contaminated with *P* elements on hybrid dysgenesis-induced polygenic mutation rates, we have constructed *X* chromosome lines from crosses of inbred P and M strains that allow us to compare mutational variance of two bristle traits arising in one generation on M strain and P strain chromosomes after a dysgenic or a nondysgenic cross. In addition, the effect on mutational variance of homogenizing the genetic background by seven generations of backcrossing to either inbred P or M strains was studied for M strain *X* chromosomes that were passed through a single dysgenic or a single nondysgenic cross.

MATERIALS AND METHODS

Drosophila stocks: The gene markers and chromosomes used are described in LINDSLEY and GRELL (1968). All flies were reared on 10 ml agar-yeast-glucose medium in shell vials.

Inbred Samarkand (Sam): This is a long-established laboratory strain, originally obtained from R. MIDDLETON. It is classified as pure M in the P-M system of hybrid dysgenesis, as it contains neither complete nor defective *P* elements, and I in the I-R system of hybrid dysgenesis. A single subline of this strain was maintained for 75 generations by continuous full-sib inbreeding, followed by several generations of random mating in small mass cultures. It was further inbred by 19 generations of full-sib mating prior to the commencement of the experiments described below, then inbreeding was again relaxed for several generations to amplify the stock.

C(1)DX y w f/Y; Sam (Sam attached-X): An attached-*X* stock in which the free *X* and *Y* chromosomes and the autosomes were derived from the inbred Samarkand strain by ten generations of backcrossing attached-*X* females to Sam males. Male progeny of attached-*X* females receive their *X* chromosome from their father and their *Y* chromosome from their mother.

Inbred Harwich: A sample of the Harwich strain used was obtained originally from M. KIDWELL. It is a strong P strain in the P-M system of hybrid dysgenesis, with an average

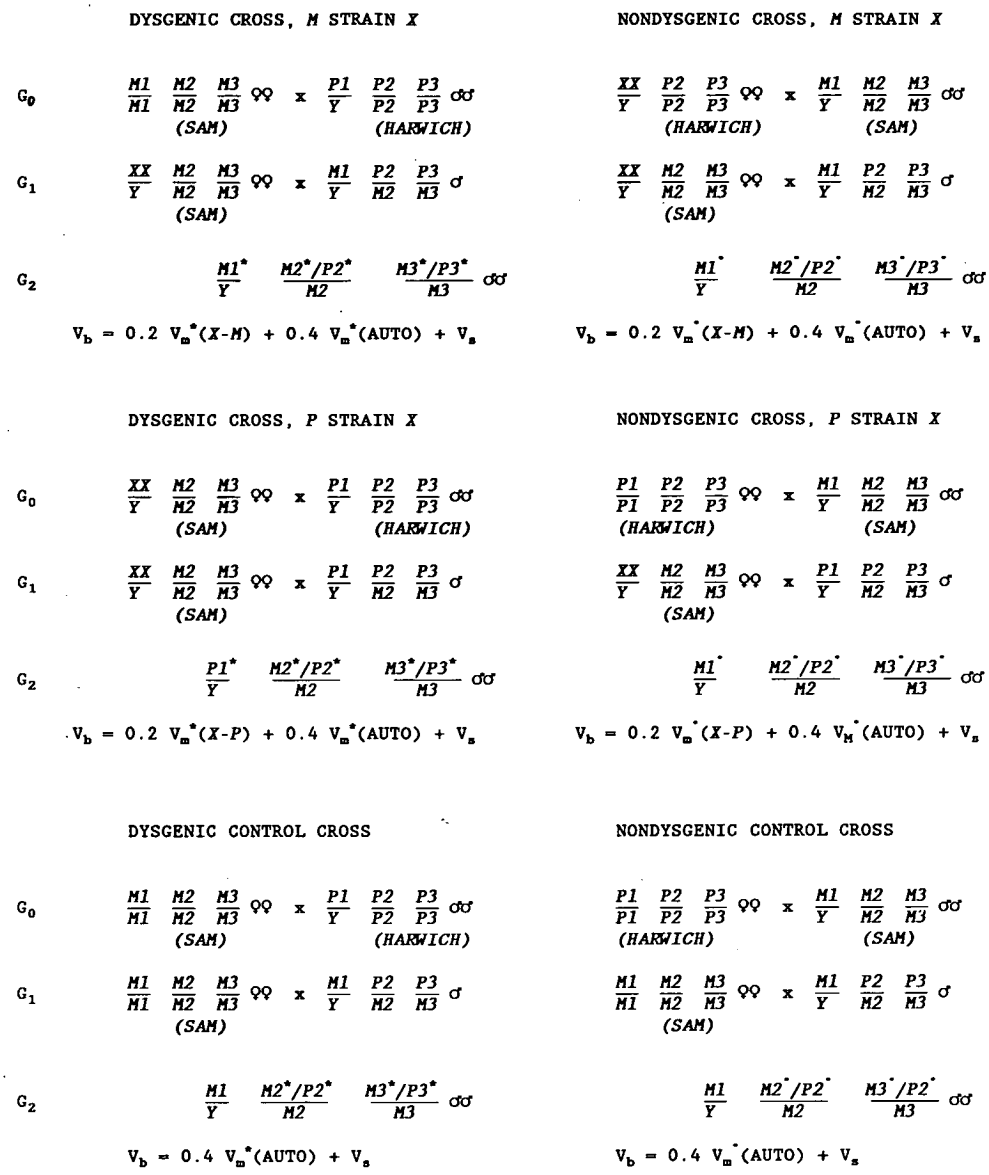

FIGURE 1.—Crossing schemes to generate P and M strain chromosome lines from dysgenic and nondysgenic crosses, and control M strain *X* chromosome lines, in a common autosomal background, and the expected composition of variance components between these lines (see text for explanation).

copy number of approximately 60 *P* elements per individual (A. E. SHRIMPTON, T. F. C. MACKAY and A. J. LEIGH BROWN, unpublished data), and is classified as I in the I-R system of hybrid dysgenesis (KIDWELL 1979). A single subline of this strain was maintained by 41 generations of continuous full-sib inbreeding, which was relaxed for several generations to build up numbers before it was used in the experimental crosses.

C(1)DX y w f/Y; Harwich (Harwich attached-X): An attached-*X* stock in which the free *X* and *Y* chromosomes and the autosomes were derived from the inbred Harwich population by ten generations of backcrossing attached-*X* females to Harwich males. (As these were initially dysgenic crosses, the first five backcross generations were reared at 20° to avoid sterility.)

Derivation of *X* chromosome lines: The following crosses were designed to estimate the mutational variance of quantitative traits induced on inbred P strain and M strain *X* chromosomes passed through single dysgenic (M × P) or nondysgenic (P × M) crosses, while maintaining a common autosomal background. All crosses were made at 20°. These crosses are depicted diagrammatically in Figure 1.

Dysgenic cross, M strain X chromosome (N = 100 lines): Inbred Sam (M) females were crossed to inbred Harwich (P) males in small mass matings of eight males and eight females. Each chromosome line was founded by a single dysgenic F_1 male, which was crossed to five Sam attached-*X* females. F_2 males inherit copies of their father's M strain-derived *X* chromosome which was potentially contaminated with *P* elements from the dysgenic cross. The autosomal background of the F_2 males segregates for Sam (75%) and Harwich (25%) alleles and may also be heterozygous for autosomal mutations induced by the dysgenic cross. A second replicate of $N = 148$ lines was made of this cross (see below).

Nondysgenic cross, M strain X chromosome (N = 94 lines): Attached-*X* Harwich (P) females were crossed to inbred Sam (M) males in small mass matings of eight males and eight females. Each chromosome line was founded by a single F_1 nondysgenic male, which was crossed to five Sam attached-X females. F_2 males inherit copies of their father's M strain-derived *X* chromosome that passed through a nondysgenic cross, in an autosomal background that segregates for Sam (75%) and Harwich (25%) alleles.

Dysgenic cross, P strain X chromosome (N = 97 lines): At-

tached-*X* Sam (M) females were crossed to inbred Harwich (P) males in small mass matings of eight males and eight females. Each chromosome line was founded by a single dysgenic F_1 male crossed to five Sam attached-*X* females. F_2 males inherit copies of their father's P strain-derived *X* chromosome which was potentially contaminated with *P* elements from the dysgenic cross, in an autosomal background identical to that of the dysgenic cross, M strain *X* chromosome, above.

Nondysgenic cross, P strain X chromosome (N = 96 lines): Inbred Harwich (P) females were crossed to inbred Sam (M) males in small mass matings of eight males and eight females. Each chromosome line was founded by a single nondysgenic F_1 male crossed to five Sam attached-*X* females. F_2 males inherit copies of their father's P strain-derived *X* chromosome that passed through a nondysgenic cross, in an autosomal background identical to that of the nondysgenic cross, M strain *X* chromosome, above.

Dysgenic control (N = 126 lines): Inbred Sam (M) females were crossed to inbred Harwich (P) males in small mass matings of eight males and eight females, and single dysgenic F_1 males were crossed to five inbred Sam females to found each chromosome line. Germ-line *P* element transposition may occur on the M strain-derived *X* chromosomes of the F_2 males, but these mutations will not be expressed somatically. The autosomal background is identical to that of the dysgenic crosses, M strain *X* chromosome and P strain *X* chromosome, above.

Nondysgenic control (N = 121 lines): Inbred Harwich (P) females were crossed to inbred Sam (M) males in small mass matings of eight males and eight females, and single nondysgenic F_1 males were crossed to five inbred Sam females to found each chromosome line. Mutations caused by germline *P* element transposition on the M strain-derived *X* chromosomes of the F_2 males are not expressed somatically, and the autosomal background is identical to that of the nondysgenic crosses, M strain *X* chromosome and P strain *X* chromosome, above.

Quantitative characters: Two quantitative traits, abdominal and sternopleural chaetae score, were measured on 20 F_2 males from each of the *X* chromosome lines (634 chromosome lines in total). Abdominal chaetae score was measured as the number of bristles on the posterior abdominal sternite, and sternopleural bristle score was the sum of the number of sternopleural bristles on the right and left sternopleural plates.

Expected composition of between-line variance components: The source of *X* chromosomes in each of the sets of chromosome lines described above is from a highly inbred strain, therefore the expected component of variance of bristle scores between lines contributed by the *X* chromosome is zero in the absence of mutation. However, the autosomes in each case segregate for alleles of the Sam strain and the Harwich strain, and this segregational variance (V_s) will cause divergence among line means. Additional variance between the lines may arise from new polygenic mutations affecting bristle score on the *X* chromosomes and the autosomes as the result of either dysgenic or nondysgenic crosses. Let V_m be the mutational variance per generation accruing from a dysgenic (superscript *) or nondysgenic (superscript °) cross. (Although two generations are required to synthesize each line, only *P* element-induced mutations occurring in the germ line of F_1 hybrids are expressed somatically in F_2 individuals, so mutational variation produced in one generation is measured). Let V_m(auto) refer to autosomal mutational variation and $V_m(X\text{-}M)$, $V_m(X\text{-}P)$ refer to mutational variance produced on *X* chromosomes of M and P strain origin, respectively. The expected composition of the between-line variance component (V_b) for each set of chromosome lines is then as follows.

Dysgenic cross, M strain X chromosome: The variation between lines is caused by segregational variance, V_s, and mutational variance on the *X* chromosome and autosomes as a result of the dysgenic cross ($V_m^*(X\text{-}M)$ and V_m^*(auto)). The *X* chromosome is approximately 20% of the genome, so the proportion of variance contributed by new variation on the *X* is 0.2 $V_m(X\text{-}M)$ The autosomes constitute the remaining 80% of the genome, but mutations on the autosomes are heterozygous in F_2 males, so additive mutational variation contributed by the autosomes is 0.8 V_m^*(auto)/2. Thus $V_b = 0.2\ V_m^*(X\text{-}M) + 0.4\ V_m^*(\text{auto}) + V_s$.

Nondysgenic cross, P strain X chromosome: These lines differ from dysgenic cross, M strain *X* chromosome, only in the direction of the original interstrain cross. Therefore $V_b = 0.2\ V_m^\circ(X\text{-}M) + 0.4\ V_m^\circ(\text{auto}) + V_s$.

Dysgenic cross, P strain X chromosome: These lines differ from the dysgenic cross, M strain *X* chromosome, only in the source of *X* chromosome. Therefore $V_b = 0.2\ V_m^*(X\text{-}P) + 0.4\ V_m^*(\text{auto}) + V_s$.

Nondysgenic cross, P strain X chromosome: These lines differ from the dysgenic cross, P strain *X* chromosome, only in the source of *X* chromosome. Therefore $V_b = 0.2\ V_m^\circ(X\text{-}P) + 0.4\ V_m^\circ(\text{auto}) + V$.

Dysgenic control: The *X* chromosomes of the F_2 males of this cross come directly from the inbred Sam (M) strain, and therefore contribute no variation between lines. However, the autosomes are heterozygous for any mutations occurring as a result of the dysgenic cross. Therefore $V_b = 0.4\ V_m^*(\text{auto}) + V_s$.

Nondysgenic control: These lines differ from the dysgenic control in the direction of the initial cross. Therefore $V_b = 0.4\ V_m^\circ(\text{auto}) + V_s$.

Estimation of mutational variance: Components of variance between lines for the two quantitative traits ($\hat{V}_b$) were estimated by the least squares and maximum likelihood analysis of variance program of W. R. HARVEY. Estimates of mutational variance ($\hat{V}_m^*(X\text{-}M)$, $\hat{V}_m^*(X\text{-}P)$, $\hat{V}_m^\circ(X\text{-}M)$, $V_m^\circ(X\text{-}P)$) were obtained by equating observed and expected between-line variance components, and solving the simultaneous equations for the dysgenic and nondysgenic crosses described above with the average between-line variance of the control crosses.

Evolution of mutational variance: At generation F_3, dysgenic, M strain *X* chromosome lines and nondysgenic, M strain chromosome lines were chosen at random to be backcrossed to either inbred Sam (M) or Harwich (P) strains. For seven generations, five males from each line were crossed to five virgin females from either the Sam attached-*X* or Harwich attached-*X* strain. At the end of the backcrossing period the autosomes are expected to be nearly isogenic (greater than 99% identity (FALCONER 1981)) with the inbred backcross parent strain, although there may be residual heterozygosity remaining from autosomal mutations occurring during backcrossing. The variance between these lines for the bristle traits therefore is due mainly to accumulated mutational variation on the initially isogenic *X* chromosomes. Comparison of the variance components between lines for the two traits from the backcross lines to the between-line variance components after a single dysgenic or nondysgenic cross will indicate to what extent the mutational variance has altered over time, and will provide data on mutational heritability comparable to that of MACKAY (1987). A total of 28 nondysgenic, M strain *X* chromosomes were backcrossed to Sam attached-*X* females, and 30 to Harwich attached-*X* females; 46 dysgenic M strain *X* chro-

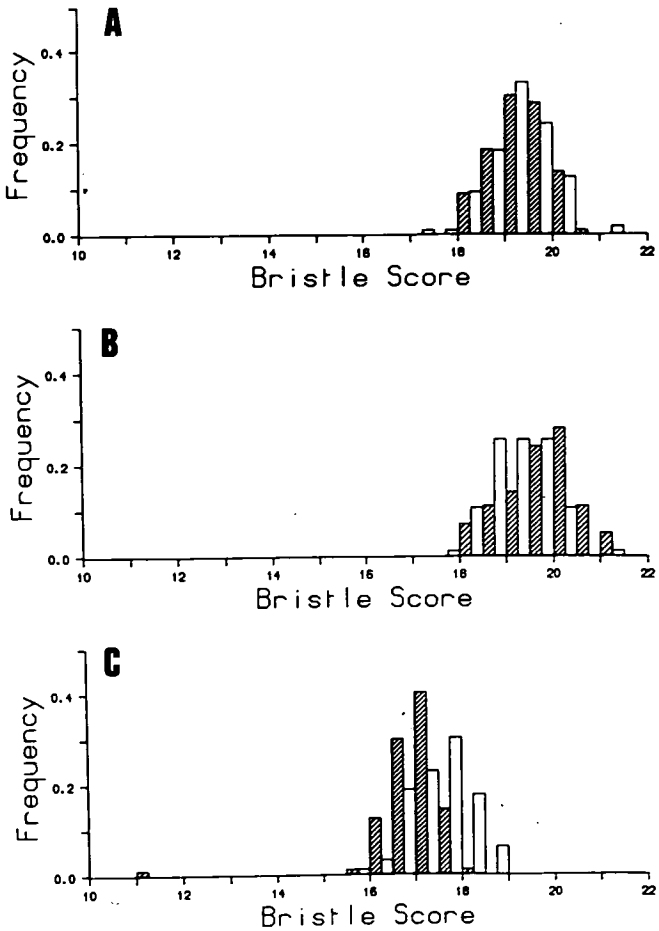

FIGURE 2.—Distributions of *X* chromosome line means for abdominal bristle score. Dysgenic (cross-hatched bars) and nondysgenic (open bars) control M strain *X* chromosomes are shown in panel A, dysgenic and nondysgenic M strain *X* chromosomes in panel B, and dsygenic and nondysgenic P strain *X* chromosomes in panel C.

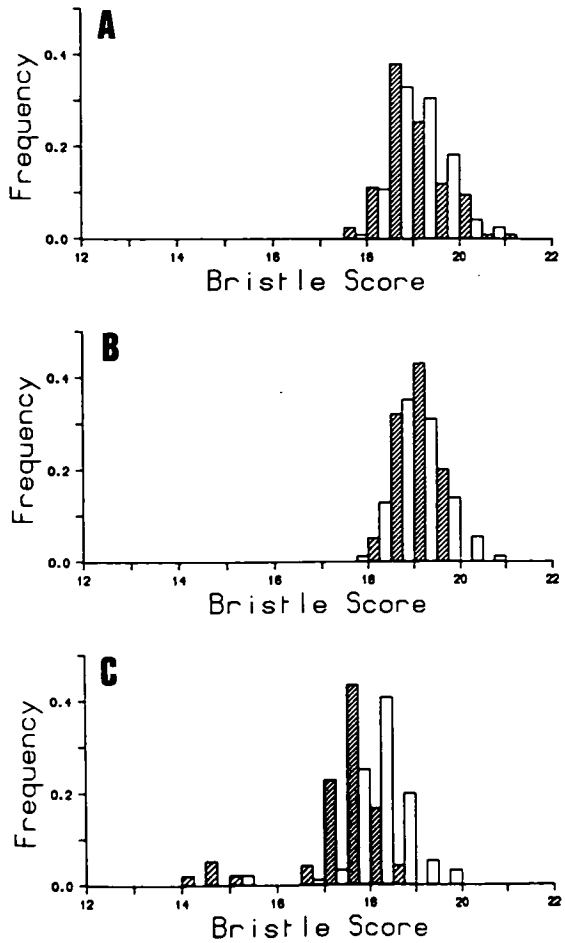

FIGURE 3.—Distribution of *X* chromosome line means for sternopleural bristle score. Dysgenic (cross-hatched bars) and nondysgenic (open bars) control M strain *X* chromosomes are shown in panel A, dysgenic and nondysgenic M strain *X* chromosomes in panel B and dysgenic and nondysgenic P strain *X* chromosomes in panel C.

mosomes were backcrossed to Sam attached-*X* females and 39 to Harwich attached-*X* females.

RESULTS

The distributions of sternopleural and abdominal bristle score for chromosome lines derived from inbred M and P strain *X* chromosomes passed through a single dysgenic cross, a single nondysgenic cross, or a control cross, and recovered in a common autosomal background, are shown in Figures 2 and 3. The statistical analyses of these distributions are given in Table 1.

Population means: The overall means of the dysgenic, nondysgenic, and control populations of M strain *X* chromosomes differ little for either trait. However, average bristle scores of the P strain *X* chromosomes are lower for dysgenic than nondysgenic populations, and also lower than mean bristle scores of M strain *X* chromosome populations. The latter difference is because inbred Harwich (P) has lower average bristle scores for both traits than inbred Sam (M), and alleles contributing to the difference are present on the *X* chromosome. The magnitude of the effect contributed by M-derived *vs.* P-derived *X* chromosomes can be calculated by comparing mean bristle scores of F_1 males from dysgenic and nondysgenic crosses of inbred Harwich and Sam with M strain *X* chromosomes (mean abdominal bristle score, 20.0; mean sternopleural bristle score, 19.0) to average bristle scores of F_1 males from dysgenic and nondysgenic crosses of these strains with P strain *X* chromosomes (mean abdominal bristle score, 18.1; mean sternopleural bristle score, 17.6). This difference of 1.9 abdominal and 1.4 sternopleural bristles contributed by M and P strain *X* chromosomes is consistent with that observed between the populations of F_2 males with P *X* chromosomes and those with M *X* chromosomes. The significant ($P < 0.02$) difference of 0.6 abdominal and 0.7 sternopleural bristles between P strain *X* chromosomes derived from nondysgenic compared to dysgenic crosses appears to be caused by several lines with extremely low bristle scores in the

TABLE 1

Statistics for M and P *X* chromosome lines derived from a single dysgenic (D), nondysgenic (ND) or control (C) cross

Cross	Source of *X*	No. of lines	Bristle trait	$\bar{X}$ (SE)	$\hat{V}_b$ (SE)	$\hat{V}_w$	Skewness (SE)
ND, C	M	121	A	19.3 (0.06)	0.133 (0.046)	4.24	−0.05 (0.22)
			S	19.1 (0.5)	0.201 (0.041)	2.25	0.62 (0.22)
D, C	M	126	A	19.3 (0.05)	0.130 (0.045)	3.78	−0.20 (0.22)
			S	19.1 (0.06)	0.288 (0.049)	2.02	0.95 (0.22)
ND	M	94	A	19.7 0.07)	0.143 (0.055)	4.33	0.27 (0.25)
			S	19.6 (0.06)	0.165 (0.043)	2.41	0.55 (0.25)
D	M	(1) 100	A	19.8 (0.08)	0.363 (0.082)	4.31	−0.63 (0.24)
			S	19.1 (0.04)	0.032 (0.022)	2.35	−0.08 (0.24)
		(2) 148	A	19.2 (0.05)	0.124 (0.038)	4.00	−0.36 (0.20)
			S	19.6 (0.04)	0.064 (0.021)	2.21	0.15 (0.20)
ND	P	96	A	17.5 (0.06)	0.206 (0.057)	3.57	−1.01 (0.25)
			S	18.1 (0.07)	0.321 (0.065)	2.54	−2.47 (0.25)
D	P	97	A	16.9 (0.08)	0.403 (0.077)	2.68	−10.19 (0.25)
			S	17.4 (0.10)	0.834 (0.134)	2.09	−7.11 (0.25)

Given are the means ($\bar{X}$), between-line ($\hat{V}_b$) and within-line ($\hat{V}_w$) components of variance, and measure of skewness (SNEDECOR and COCHRAN 1971) for two bristle traits, abdominal (A) and sternopleural (S) bristle counts. Standard errors (SE) are in brackets.

P *X* chromosome dysgenic population. One line has a mean abdominal bristle score of 11, three standard deviations below the population mean, and nine lines have mean sternopleural bristle scores over one standard deviation less than the overall mean (Figure 1c). However, the mean of both bristle traits remains lower for dysgenic than nondysgenic P strain *X* chromosomes even if the extreme lines are excluded (differences of 0.52 abdominal and 0.51 sternopleural bristles), but these differences in mean are not statistically significant.

Variance components: Estimates of between- and within-line components of variance of abdominal and sternopleural bristle score for the *X*-chromosome populations are given in Table 1. The expected between-line component of variance from chromosomes is zero in the absence of mutation, because the *X* chromosomes are from inbred lines. However, the segregating autosomal background will contribute variance between lines; this is estimated from the dysgenic and nondysgenic control populations to be 3% of the total variance for abdominal bristles, and 10% of the total variance for sternopleural bristles. There is no clear heterozygous effect of P-induced autosomal polygenic mutations: between-line variance components are similar for the dysgenic and nondysgenic control populations.

Therefore P-induced *X*-linked polygenic mutational variation can be detected by comparing the between-line components of variance of the populations with different *X* chromosome origin and treatment to the basal level of between-line segregational variance, estimated from the average between-line variance of dysgenic and nondysgenic control populations. For the population of M strain *X* chromosomes passed through one nondysgenic cross, this comparison shows no significant difference in between-line variance for either bristle trait. In contrast, the first replicate of the M strain *X* chromosomes derived from dysgenic crosses gave a nearly threefold increase in the between-line component of variance of abdominal bristle score, suggesting the occurrence of dysgenesis-induced mutations affecting this trait, but a substantial reduction in the between-line sternopleural bristle variance. To determine whether this reduction in variance was a real effect or caused by sampling, a second replicate was made of dysgenic M strain *X* chromosomes. Again, the between-line variance of sternopleural bristle score was reduced compared to the control cross; in this case the between-line variance of abdominal bristle score was not different from the control. P strain *X* chromosome lines derived from dysgenic crosses had between-line variances for both bristle traits elevated greater than three times the control value. P strain *X* chromosome lines derived from nondysgenic crosses also exhibited increased between-line components of variance, but less so than those that had undergone dysgenic crosses (a 60% increase for abdominal, and 30% increase in sternopleural bristle variance compared to the control). Inasmuch as the variance components for the bristle traits are diagnostic of the occurrence of P-induced bristle mutations, this analysis indicates (1) P strain *X* chromosomes are more mutable than M strain *X* chromosomes, and (2) dysgenic crosses are more effective in generating mutations than nondysgenic crosses, but mutations arising from nondysgenic crosses involving P strain target chromosomes are not negligible.

Mutational variance: Mutational variances ($\hat{V}_m$) for

TABLE 2

Mutational variance and mutational heritability

Cross	X chromosome	Bristle trait	$\hat{V}_m$	$\hat{h}^2_m$
Dysgenic	M[a]	A	0.561	0.144
		S	(−)	(−)
	P	A	1.356	0.289
		S	2.945	0.652
Nondysgenic	M	A	0.057	0.017
		S	(−)	(−)
	P	A	0.371	0.100
		S	0.380	0.195

[a] Average of replicates (1) and (2). Estimates of mutational variance ($\hat{V}_m$) and mutational heritability [$\hat{h}^2_m = \hat{V}_m/(\hat{V}_m + \hat{V}_e)$] generated by dysgenic or nondysgenic crosses in P or M strain backgrounds are given for abdominal (A) and sternopleural (S) bristle score. (−) indicates a negative estimate.

the two quantitative traits, abdominal and sternopleural bristle number, were estimated for each of the four populations of *X* chromosomes from the difference between the observed between-line components of variance and the average control between-line variance for each trait, multiplied by a factor of five to scale the *X* chromosome estimate to the whole genome. Mutational heritabilities (h^2_m) were estimated as $\hat{V}_m/(\hat{V}_m + \hat{V}_e)$, where $\hat{V}_e$, the environmental variance, was calculated from the average within-line variance of the two characters in the Harwich (P) and Sam (M) inbred strains (see Table 3). For abdominal bristle score, $\hat{V}_e = 3.33$ and for sternopleural bristle score, $\hat{V}_e = 1.57$. The estimates are given in Table 2. In general, a single generation of *P* element mutagenesis generates exceptionally high levels of variation for bristle traits, leading to heritabilities of a magnitude typically observed in natural populations (FALCONER 1981). P-induced mutational variances are greater for P strain than M strain chromosomes, and greater for dysgenic than nondysgenic crosses. Note that whenever there is appreciable mutational heritability for a trait, the distribution of that trait is significantly negatively skewed (compare Tables 1 and 2), indicating the mutations predominantly reduce bristle score.

To test for the continued production of mutational variance for the two bristle traits after the initial dysgenic or nondysgenic cross, dysgenic and nondysgenic M *X* chromosomes were backcrossed to inbred M and P strain females for seven generations. The first backcross generation used F_3 males from the *X* chromosome lines described above, giving nine generations over which mutations could accumulate. By the end of seven backcross generations the autosomes are expected to be over 99% isogenic with the inbred strains (FALCONER 1981), so the only source of variation between lines is mutational variance on the *X*. The estimates of V_m from these lines are simply the between-line components of variance, scaled by a factor of five to be comparable to the whole genome, and divided by the number of generations over which mutations could arise. A conservative estimate is to assume mutations occur at an equal rate for nine generations, but it is possible that P mutagenesis happens only in the first few backcross generations. Since the pattern of P transposition is unknown in these experiments, estimates of V_m are given in Table 3 assuming mutagenesis for two different time periods. If *P* element mutagenesis occurs for five generations, $\hat{V}_m = \hat{V}_b$; the estimates for nine generations are in the column labeled $\hat{V}_m$.

TABLE 3

Statistics for M strain *X* chromosomes backcrossed to inbred P and M strains

Initial cross	BX strain	*N*	Bristle trait	$\hat{V}_b$	$\hat{V}_w$	$\hat{V}_m$	$\hat{h}^2_m$ (9)	$\hat{h}^2_m$ (5)
Dysgenic	M	46	A	0.215	3.29	0.119	0.035	0.061
			S	0.125	1.78	0.069	0.042	0.074
	P	39	A	0.607	3.62	0.337	0.092	0.154
			S	0.201	1.75	0.112	0.067	0.113
Nondysgenic	M	28	A	0.210	2.88	0.117	0.034	0.059
			S	0.093	1.39	0.052	0.032	0.056
	P	30	A	0.390	3.53	0.217	0.061	0.105
			S	0.219	1.37	0.122	0.072	0.122

Between- ($\hat{V}_b$) and within- ($\hat{V}_w$) line variance components, mutational variance ($\hat{V}_m$) and mutational heritability [$\hat{V}_m/(\hat{V}_m + \hat{V}_e)$] for abdominal (A) and sternopleural (S) bristle score are given for inbred M strain *X* chromosomes passed through a single dysgenic or nondysgenic cross, then backcrossed (BX) for seven generations to the inbred P or M strain. *N* is the number of chromosome lines of each cross. The two values of $\hat{h}^2_m$ were calculated assuming different numbers of generations of mutation accumulation (see text for explanation).

The between-line variances for abdominal and sternopleural bristle score contributed by M strain *X* chromosomes derived from a single dysgenic cross were 0.112 and negative, respectively [average of replicates (1) and (2)]; for nondysgenic M strain *X* chromosomes the estimates were not different from zero–0.011 and negative. The between-line variance of dysgenic and nondysgenic M strain chromosomes backcrossed to inbred P or M strains increased compared to the initial values (Table 3), which is indicative of further mutagenesis during backcrossing.

Estimates of mutational heritability were calculated assuming nine [$\hat{h}^2_m(9)$] or five [$\hat{h}^2_m(5)$] generations of potential mutagenesis, and using the estimates of environmental variance given above. Although the estimates assuming fewer generations of mutation accumulation were 70% larger than the most conservative estimates, both displayed the same patterns. (1) Mutational heritabilities were similar for both bristle traits. (2) The initial difference in $\hat{h}^2_m$ between *X* chromosomes subjected to one dysgenic or nondysgenic cross disappeared after seven backcross generations.

TABLE 4

Bristle mutant lines

		Abdominal bristles			Sternopleural bristles		
Origin	Line	$\bar{X}$	t	SD	$\bar{X}$	t	SD
DP	98	16.8	−0.46	−0.10	15.0	−6.32*	−1.41
	103	17.1	0.31	0.07	15.2	−5.92*	−1.32
	112	16.7	−0.59	−0.13	14.9	−6.71*	−1.50
	114	17.1	0.43	0.10	14.7	−7.23*	−1.62
	126	16.7	−0.71	−0.16	14.9	−6.58*	−1.47
	146	11.5	−13.98*	−3.13	14.9	−6.71*	−1.50
	159	17.2	0.56	0.13	14.0	−8.94*	−2.00
	164	17.2	0.56	0.13	14.8	−6.84*	−1.53
	187	16.5	−1.11	−0.25	14.4	−7.89*	−1.76
NDP	108	17.0	−1.17	−0.26	15.5	−7.12*	−1.59
	149	17.5	−0.14	−0.03	15.1	−8.18*	−1.83
NDC	19	10.3	−19.44*	−4.35	11.0	−23.11*	−5.17

* $P \ll 0.001$.

Mean ($\bar{X}$) abdominal and sternopleural bristle score, t-statistic, and deviation from population mean in standard deviation units (SD) for X chromosome lines with putative bristle mutations. These originated on P strain X chromosomes after one dysgenic cross (DP), P strain X chromosomes after one nondysgenic cross (NDP), and on a nondysgenic control (NDC) M strain X chromosome during maintenance.

(3) Mutational heritabilities from X chromosomes backcrossed to a P strain were twice that of X chromosomes backcrossed to an M strain.

Mutant effects: Twelve lines have been identified with extreme bristle phenotypes on the basis of the deviation of their scores from the overall population mean. Of these, nine were observed on P strain X chromosomes after a single dysgenic cross, two on P strain X chromosomes after a single nondysgenic cross, and one on an M strain X chromosome from the nondysgenic control population at generation 4 of maintenance. The mean sternopleural and abdominal bristle score based on a sample size of 20 individuals, deviations from the overall population mean in standard deviation units, and the Student's t statistic for the test of significance of the difference in mean are given in Table 4. Only sternopleural bristle score is affected for ten of the mutant lines, while both traits are extreme for the remaining two lines. In all cases the direction of the mutant effect is to decrease bristle score. It is interesting to note that the lines with sternopleural bristle scores averaging 1.6 standard deviations below the mean were identified as mutant with reference to their base population, but otherwise their scores are well within the wild-type range. They are therefore true "polygenic" mutations. The lines with abdominal and sternopleural bristle scores greater than three standard deviations below the mean are, however, quite extreme in comparison to wild-type variation.

Assuming each line bears an independent P-induced bristle mutation, the polygenic mutation rate on P X chromosomes from one dysgenic cross is 9.3%, and from one nondysgenic cross is 2%. However, the ten lines for which only sternopleural bristle score was affected all have similar scores, and it is possible they all result from a common event involving a resident P element on the target P chromosome situated near a locus affecting bristle score. The two lines deviant for both traits have mutant phenotypes distinct from the other ten lines. Genetic and cytological mapping experiments are in progress to determine allelism of these mutants, and whether they can be associated with P element insertions or deletions. The bristle phenotype of line number 146 (see Table 4) is unstable and has reverted to both more extreme and wild-type phenotypes, which suggests that this mutation at least is caused by a P element insertion.

DISCUSSION

The ability of the P-M system of hybrid dysgenesis in *D. melanogaster* to generate mutations affecting quantitative characters was tested by measuring the *de novo* production of variation for two bristle traits (abdominal and sternopleural bristles) between X chromosomes of inbred P and M strains passed through a single dysgenic (M × P) or nondysgenic (P × M) cross, in a common autosomal background. Dysgenic crosses are capable of inducing polygenic mutational variance, given by the mutational heritability as a proportion of the environmental variance, at levels equivalent to heritabilities of these traits from natural populations (FALCONER 1981). This magnitude of mutagenesis is unprecedented; a single generation of exposure to P element movement is 100 times more powerful than 1000 r X-rays in inducing variation for abdominal bristle count (MACKAY 1987). The sole exception is the variance of sternopleural bristle score between dysgenic M strain X chromosomes, which was less than the control between-line variance of this trait. A possible explanation, other than the obvious interpretation of sampling error as a cause of the difference, is that new mutants for this trait were lethal, and therefore not recovered. Mutational heritabilities of the bristle traits were about eight times greater when P strain X chromosomes were used as targets; this is not surprising because P-induced mutations on P chromosomes can occur by excision as well as insertion. Eight of the nine P strain X chromosomes showing extreme bristle scores had similar phenotypes, and could have been generated by excision of a P element near a locus affecting bristle score. Although nondysgenic crosses of M strain X chromosomes did not give rise to detectable polygenic mutations, nondysgenic crosses of P strain X chromosomes did, suggesting that the repression of P transposition is not complete in this situation.

Maintenance by backcrossing to inbred M or P

strains of originally isogenic M strain chromosomes that were passed through one dysgenic or one nondysgenic cross generates further mutational variance for bristle traits. Additional mutagenesis for the case of the M strain backcross parent is explicable because these are dysgenic crosses. The autosomal background of the F_3 males used at the first backcross generation, whether they were derived from dysgenic or nondysgenic crosses, potentially contains intact *P* elements, and so the opportunity exists for further hybrid dysgenesis-induced P transposition on crossing to M cytotype females. However, the evolution of polygenic mutational variance among M strain *X* chromosomes backcrossed to inbred P strain females, which was twice as great as the variance induced by backcrossing to M strain females, is less easy to understand. These backcrosses are nondysgenic; by what mechanism could *P* elements be mobilized by nondysgenic interstrain crosses? One explanation might be that the inbred P strain used was altered from the original Harwich strain in terms of its ability to repress intrastrain P transposition, and so was unstable. TORKAMANZEHI, MORAN and NICHOLAS (1988) showed their subline of Harwich had high levels of intrastrain ovarian dysgenesis at 29°, suggestive of *P* element mobility within this strain. It is also possible that another hybrid dysgenesis system is operating in addition to P-M dysgenesis. The Harwich and Samarkand strains used are classified as I in the I-R hybrid dysgenesis system, but their status with regard to *hobo* dysgenesis (BLACKMAN *et al.* 1987; YANNOPOULOS *et al.* 1987) is not known.

These results are comparable to those of MACKAY (1986, 1987) who demonstrated that M strain second chromosomes passed through initial dysgenic and nondysgenic crosses evolved similar levels of polygenic mutational variance for a number of quantitative traits after eight backcrosses to a Harwich-derived P strain. This effect was only detectable in comparison to variation for the traits among control M strain chromosomes which necessarily had a different genetic background. TORKAMANZEHI, MORAN and NICHOLAS (1988) and PIGNATELLI and MACKAY (1989) found accelerated responses to artificial selection for bristle traits in lines started from nondysgenic interstrain crosses, which they interpreted in terms of new P-induced mutations affecting the bristle traits. The observations of A. E. SHRIMPTON, T. F. C. MACKAY and A. J. LEIGH BROWN (unpublished data) of equal amounts of P transposition in the dysgenic and nondysgenic selection lines of MACKAY (1985) lend support to this interpretation. The production of P-induced polygenic mutations from nondysgenic as well as dysgenic crosses confounds the estimation of mutational variation unless accumulation of mutations is restricted to a single generation, but this problem should not detract from the overall conclusion that *P* elements are capable of inducing very high levels of variation for quantitative traits.

The effects of all P-induced mutations affecting bristle score observed on *X* chromosomes in this experiment are large, and decrease the value of the traits, giving highly negatively skewed distributions of mutant effects. The nine dysgenic P strain *X* chromosome mutants are responsible for all of the new variation between lines for sternopleural and abdominal bristle score; removal of these lines from the analysis drops the between-line component of variance well below the basal control value for each trait. When a few mutations cause most of the new polygenic variation, estimates of mutational variance are expected to be noisy, and will be large when a sample contains a new mutant and negligible otherwise. This may partly explain the large variance in response among replicate selection lines started from dysgenic and nondysgenic crosses (MACKAY 1985; TORKAMANZEHI, MORAN and NICHOLAS 1988; PIGNATELLI and MACKAY 1989). Verification of the generality of the observation that new mutations primarily decrease bristle number must await the creation of a larger sample of bristle mutations and genetic and cytological analysis of the ones described above. *P* elements are capable in general of causing a spectrum of mutant effects resulting from increasing or decreasing the amount or altering the timing or tissue-specific expression of relevant gene products (ENGELS 1988). *P* element mutagenesis thus offers exciting prospects for identifying and mapping quantitative trait loci in Drosophila, and understanding the genetic and molecular basis of quantitative variation.

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